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SPECIALIZATION OF PATHOGENICITY IN ERYSIPHE GRAMINIS ON WILD AND CULTIVATED GRASSES[1]

JOHN R. HARDISON[2]

(Accepted for publication July 20, 1943)

INTRODUCTION

Since the discovery of pathogenic specialization in *Erysiphe graminis* DC. by Marchal (12), many authors have contributed to our knowledge of the subject. Seven physiologic varieties of the fungus have been described on the basis of restriction of infection to the species of a given genus of grasses. Some of these physiologic varieties have been further subdivided into physiologic races distinguished by their ability to infect certain species or agronomic varieties of species within a host genus. Powdery mildew of the cereals has been most comprehensively studied, but in most instances only a few grasses were tested in host range studies. Blumer (1) lists the pathogen on 76 species of 32 genera of the Gramineae, but cultures of the fungus from only 7 genera have been studied intensively. The present investigation was undertaken primarily to determine the infection range of the fungus from other grasses.

LITERATURE REVIEW

The first cross-inoculation experiments with the Erysiphaceae were reported by Wolff (33). His inoculation of species of *Lupinus* and *Trifolium*, host plants of *Erysiphe communis* (*E. polygoni*), with *E. graminis*, and inoculation of grasses with *E. communis* gave negative results, according to Salmon (22). Wolff (32), however, reported successful infection of wheat with ascospores of *Erysiphe graminis* from perithecia formed on wheat.

Marchal (12) reported the first positive infection from cross-inoculation experiments with *Erysiphe graminis*, and, on the basis of host specialization, distinguished the following 7 specialized forms: *tritici* on species of *Triticum*, *hordei* on species of *Hordeum*, *secalis* on species of *Secale*, *avenae* on species of *Avena* and *Arrhenatherum elatius*, *poae* on species of *Poa*, *agropyri* on *Agropyron* species, and *bromi* on species of *Bromus*.

Erysiphe graminis tritici, as distinguished by Marchal, infected *Triticum polonicum*, *T. spelta*, *T. turgidum* but not *Agropyron* spp., *Avena sativa*,

[1] Part of a thesis submitted to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany. Paper No. 821, from the Department of Botany.

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The writer is indebted to Dr. E. B. Mains for his helpful criticism and advice during the course of the investigation, and to Dr. George W. Fischer for constructive criticism and suggestions in the preparation of the manuscript. Grateful acknowledgment is made for the many accessions of grass species and cereal varieties received from the following: Dr. George W. Fischer, Dr. W. L. Brown, Dr. E. B. Mains, Mr. Walter Kleinschmidt and to the Regional Nursery Division, Soil Conservation Service, Pullman, Washington. Indebtedness is also acknowledged to Mr. J. R. Swallen for checking the identity of the majority of grasses used.

Bromus spp., *Hordeum vulgare*, *Poa* spp., *Secale cereale*, *Triticum dicoccum*, *T. durum*, or *T. monococcum*. Marchal (13) reported further that ascospores produced in perithecia obtained from *Triticum vulgare* infected *T. vulgare* but not *Agropyron caninum*, *Avena sativa*, *Hordeum vulgare*, or *Secale cereale*.

Marchal's results were confirmed by Salmon (23), who states that wheat mildew could not infect *Agropyron repens*, *Avena sativa*, *Hordeum vulgare*, or *Secale cereale*. Salmon (28) transferred wheat mildew to young plants of *Hordeum silvaticum*. Reed (16) pointed out that the *Index Kewensis* lists *Hordeum silvaticum* as a synonym of *Elymus europaeus*. Reed further states that plants resulting from a wheat × rye hybrid were highly resistant to *Erysiphe graminis tritici* and *E. graminis secalis*. Information on the reaction of wheat varieties is given by Reed (17), and, quoting from unpublished results (18), he states that wheat mildew infected different species of *Aegilops*.

Reed (19) transferred wheat mildew to *Aegilops aristata*, *A. aucheri*, *A. cylindrica*, *A. ligustica*, *A. speltoides*, *A. squarrosa* L., *A. triaristata*, *Triticum dicoccum* Schrank., *T. durum* Desf., *T. freycenetii* Host, *T. meyeri*, *T. monococcum* L., *T. polonicum* L., *T. spelta* L., *T. thaoudar* Reut., *T. tumonia* Schrad, *T. turgidum* L., and *T. vulgare* Vill. Both resistant and susceptible varieties were found in the *Triticum* species. There was no infection of *Aegilops ovata* L., *A. triticoides*, *Avena sativa* L., *Brachypodium distachyum* Beauv., *Hordeum vulgare* L., *Secale cereale* L., or *Triticum dicoccum*.

Mains (10) distinguished 2 physiologic races within *Erysiphe graminis tritici*, and discussed the reactions of many varieties of several species of *Triticum*. The following grasses proved very resistant to physiologic race 1: *Agrostis alba* L., *A. perennans* (Walt.) Tuckerm., *Agropyron cristatum* J. Gaert., *A. repens* (L.) Beauv., *Arrhenatherum elatius* (L.) Beauv., *Bromus ciliatus* L., *B. erectus* Huds., *B. hordeaceus* L., *B. japonicus* Thunb., *B. mollis* L., *B. pumpellianus* Schribn., *B. secalinus* L., *B. tectorum* L., *Festuca elatior* L., *F. rubra* L., *Elymus canadensis* L., *E. condensatus* Presl., *E. striatus* Willd., *E. virginicus* L., *Hordeum jubatum* L., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt., *Hystrix hystrix* Millsp., *Secale montanum* Guss., and *Sitanion hystrix* (Nutt.) J. C. Smith. Also to physiologic race 1, resistant and susceptible varieties were reported in *Triticum vulgare* Vill., *T. spelta* L., *T. polonicum* L., *T. compactum* Host, *T. durum* Desf., *T. turgidum* L., *T. dicoccum* Schrk., *T. stramineum*, and only resistant varieties were found in *T. monococcum* L., *T. dicoccoides*, *T. persicum*, and *T. timopheevi*. To physiologic race 2 *Aegilops squarrosa* L., and *A. ventricosa* Tausch. were moderately resistant, *A. crassa* Boiss., *A. cylindrica* Host, *A. ovata* L. and *A. triuncialis* L. were very resistant, and several strains of *Secale cereale* and *S. montanum* exhibited no signs of infection.

Schlieting (29) distinguished 6 races of *Erysiphe graminis tritici* in Germany and another race that in all probability is undescribed. These

differ from the two races of Mains. Rosenthiel (21) reported 5 races of *E. graminis tritici* in Germany, but the differential varieties of Schlicting were not tested.

Marchal (12) found that *Erysiphe graminis hordei* infected *Hordeum hexastichon, H. jubatum, H. murinum, H. nudum, H. trifurcatum, H. vulgare* and *H. zeocriton,* but not *H. bulbosum, H. maritimum,* or *H. secalinum.* Also there was no infection of *Agropyron caninum, A. giganteum, A. repens, Agrostis alba, Aira caryophyllea, Alopecurus pratensis, Andropogon ischaemon, Anthoxanthum odoratum, Arrhenatherum elatius, Avena fatua, A. orientalis, A. sativa, Brachypodium sylvaticum, Briza media, Bromus arduennensis, B. macrostachys, B. mollis, B. patulus, B. racemosus, B. secalinus, B. sterilis, B. squarrosus, Cynosurus cristatus, Dactylis glomerata, Deschampsia flexuosa, Elymus arenarius, Festuca elatior, F. gigantea, F. rubra, Holcus lanatus, Kochleria cristata, Lolium perenne, Melica ciliata, Milium effusum, Phleum boehmeri, P. pratense, Poa annua, P. mulalensis, P. nemoralis, P. pratensis, P. serotina, P. trivalis, Secale cereale, Setaria viridis, Trisetum flavescens, Triticum polonicum, T. spelta,* or *T. vulgare.*

Marchal (13) states that ascospores from perithecia collected on barley were able to develop on *Hordeum distichum, H. trifurcatum, H. vulgare,* and *H. zeocriton.*

Salmon (24) reported that ascospores from barley produced no infection on *Avena sativa, Secale cereale* or *Triticum vulgare.* With conidia from *Hordeum vulgare* Salmon (25) infected *Hordeum decepiens, H. distichon, H. hexastichon, H. intermedium, H. vulgare* and *H. zeocriton.* A slight infection was obtained on *H. bulbosum* and *H. maritimum,* but no infection was produced on *Avena sativa, Hordeum jubatum, H. murinum, H. secalinum, H. sylvaticum, Triticum vulgare* or *Secale cereale.*

Reed (16), by inoculation with conidia from *Hordeum vulgare,* produced infection on *Hordeum distichon* L., *H. nudum* L., *H. steudelii* × *trifurcatum, H. tetrastichon* L., and young seedlings of *H. nodosum* L. Negative results were obtained on *Avena sativa* L., *Hordeum bulbosum* L., *H. maritimum* With., *H. jubatum* L., *Triticum dicoccum* Schrank., *T. durum* Desf., *T. vulgare* Vill. and *Secale cereale* L.

Montemartini (14) could not infect unspecified species of *Avena, Holcus,* or *Setaria* with conidia from *Hordeum murinum.*

Mains and Dietz (11) found resistant and susceptible varieties in *Hordeum deficiens, H. distichon, H. intermedium,* and *H. vulgare.* On the basis of host specialization they distinguished 5 physiologic races. In continuation of this line of investigation Tidd (30) described 2 additional races, i.e., races 6 and 7.

Honecker (4, 5, 6, 7) has described 9 races of *Erysiphe graminis hordei* for Germany. Comparison with American work is impossible, because Honecker did not use the same differential varieties of barley.

Erysiphe graminis secalis was described by Marchal (12) as infecting *Secale cereale* and *S. anatolicum* but not *Avena sativa, Hordeum vulgare* or *Triticum vulgare.* Also, Marchal inferred that there was no infection on

species of *Agropyron*, *Bromus* or *Poa*. Marchal (13) reported that ascospores of *E. graminis secalis* infected only *Secale cereale*.

Reed (15) states that conidia from *Secale cereale* infected *S. anatolicum*, *S. cereale*, and *S. montanum* but not *Avena sativa*, *Bromus mollis*, *Dactylis glomerata*, *Festuca elatior*, *F. heterophylla*, *Glyceria fluitans*, *Hordeum vulgare*, *H. jubatum*, *Lolium perenne*, *Phleum pratense*, *Poa compressa*, *P. pratensis*, *P. trivialis*, *P. nemoralis*, *Secale dalmaticum*, or *Triticum vulgare*.

Treboux (31) reported that conidia from *Secale cereale* infected *S. cereale* but not *Hordeum vulgare* or *Triticum vulgare*.

Mains (9) discovered several strains of *Secale cereale* that were resistant to *Erysiphe graminis secalis*.

Erysiphe graminis agropyri is described by Marchal (12) as occurring on species of *Agropyron*. Conidia from *Agropyron giganteum* and *A. repens* would not infect barley. This specialized variety would not infect *Avena sativa*, *Bromus* spp., *Hordeum vulgare*, *Poa* spp., *Secale cereale* or *Triticum vulgare*.

Salmon (27) states that conidia from *Agropyron repens* infected *A. caninum*, *A. repens*, and *A. tenerum*, but there was no infection of either *A. acutum* or *A. glaucum*.

Comparable results were obtained with *Erysiphe graminis avenae* by Marchal (12), Salmon (23, 27), and Reed (19, 20), with *E. graminis poae* by Marchal (12), Salmon (27), and Reed (15), with *E. graminis bromi* by Marchal (12) and Salmon (23, 26), and *Erysiphe graminis* from *Dactylis glomerata* by Salmon (27). Marchal (12) states that *Erysiphe graminis* from *Holcus lanatus* and *Festuca pratensis* would not infect *Hordeum vulgare*.

The literature records to date indicate that the physiologic varieties of *Erysiphe graminis* are mostly restricted to the species of one genus. The only exceptions have been slight infections by *Erysiphe graminis tritici* on *Hordeum sylvaticum* (*Elymus europaeus*) by Salmon (28) and on species of *Aegilops* by Reed (19), slight infection of *Arrhenatherum elatius* by *Erysiphe graminis avenae* by Marchal (12) and Reed (18) and slight infection of *Hordeum sylvaticum* (*Elymus europaeus*) by *Erysiphe graminis hordei* by Salmon (25). The following results indicate that this concept of extremely narrow specialization is mostly erroneous.

MATERIALS AND METHODS

Three hundred seventy-two accessions of the grass and cereal species[3] listed in table 1 were used. Fifty to 100 seeds were planted in 3½-inch pots containing a mixture consisting of 1 part peat, 1 part sand, 3 to 4 parts sterilized soil and a small amount of commercial fertilizer. The plants thus obtained were maintained in a separate greenhouse free from mildew and formed a stock supply of seedlings. Since the grass species vary in rate of growth, this method is necessary in order that seedlings of all species be

[3] Hitchcock (2) was followed in the classification of the grass species where possible.

available for inoculation at a given time. Some grass species must be replanted occasionally to provide a constant supply of young seedlings. For inoculation with a mildew culture 5 to 25 seedlings of each grass were transplanted to 2½-inch pots. Such sets were transferred to separate greenhouse compartments where cultures of the fungus were maintained.

The scattering or dilution method described by Mains (8) and Tidd (30) was used to obtain highly pure and probably, in most cases, single-spore cultures. This purification was done after a knowledge of pathogenicity of the bulk culture was obtained.

The writer employed the following method of inoculation: In order that the spores may not be dispersed by air currents, the plants on which a mildew culture is being maintained are placed for 24 to 48 hours in a glass-covered chamber (8). Under these conditions a large quantity of conidia accumulates. The inoculum is collected at midday by lightly tapping the leaves, allowing the masses of conidia to fall into the lower half of a Petri dish. The spores are then floated on water and poured into a 250-cc. flask. The amount of water added is estimated by the number of plants to be inoculated. The aqueous spore suspension in the flask is thoroughly agitated and applied by an atomizer. Many spores are deposited in each water droplet on the leaves. The plants are then covered for 24 hours with wet muslin cloth supported on a wire frame. This method has given uniformly good results. All leaves of all plants can be inoculated easily. On susceptible grasses 6 to 8 days after inoculation numerous infection areas are produced. It has the further advantage of not distributing spores into the air to the extent produced by the dusting method. The leaf areas resulting from new growth developed between the inoculation and recording of notes show no infection. Cloths, atomizers, and glassware used in inoculations were sterilized between cultures.

During the course of the investigation results in variance with those of previous workers were obtained with certain cultures. These results were verified under conditions that exclude the possibility of foreign contamination in the following manner. Seeds of the plants in question were planted in 3½-inch pots filled with the soil mixture described above. Glass lamp chimneys with the top end covered by two layers of light muslin-cloth were then pressed down a short distance into the soil inside the pot and the seedlings grown inside the lamp chimneys. These glass covers (8) were removed only when inoculations were performed, and then only for a few seconds while conidia were transferred by a scalpel to the leaves of plants to be inoculated. These plants were grown in a separate greenhouse free from mildew-infected plants, and the inoculum was brought into the isolated culture house in a covered Petri dish.

Notes were taken 7 to 10 days and 14 to 18 days after inoculation. Two readings proved desirable, since the inoculation sometimes produces considerable initial infection of a higher degree than will persist. The conidial suspension method provides a very heavy inoculation, and it is nearly impossible for a grass to escape the inoculation.

The five types of reaction of Mains and Dietz (11, p. 231) were employed. The symbols + and – were used to further indicate the degree of susceptibility.

Field observations of the reaction of seedling and adult plants of the grass accessions were found useful in the selection of mildew cultures for study. Many of the grasses used in this study are represented in the observational row nurseries of the Soil Conservation Service at Pullman, Washington. During the summer months of 1939, 1940, and 1941 at Pullman, the writer recorded observations on mildew reaction of many of the grasses being studied. From these data it was possible to select, efficiently, mildew cultures for study and to obtain a better understanding of greenhouse tests. A grass pathology nursery including all of the grass accessions being studied was maintained at the Botanical Garden of the University of Michigan. This nursery proved to be an excellent source for mildew cultures and for mildew observations. Mildew cultures were purposely selected for study which appeared to be different by field observations.

RESULTS

In table 1 are summarized the results of inoculations of grasses and cereals with 8 cultures of *Erysiphe graminis*. Details of significant results with each culture are discussed below.

Culture 18. On *Secale cereale*, from Yakima, Wash.

Culture 18 was almost completely restricted to *Secale cereale*. The only other infection was a very resistant reaction on *Agropyron spicatum*. The infection results reported for many grass species represents the first large scale, negative evidence yet presented for the fungus from rye.

Culture 2. On *Triticum aestivum*, from Ann Arbor, Mich.

This culture produced very unusual results. Infection was produced on a number of grass species of several genera, all in the tribe Hordeae. A very susceptible reaction was recorded on *Aegilops crassa*, *A. cylindrica*, *Agropyron striatum*, *Elymus condensatus* and *E. junceus*, and a moderately susceptible reaction was exhibited on *Aegilops triuncialis*, *Agropyron inerme*, *A. spicatum*, *Elymus canadensis*, *E. dahuricus*, *E. triticoides*, *E. glaucus*, *E. sibiricus* and *Sitanion hystrix*. Also, a moderately resistant reaction was recorded on *Agropyron spicatum*, *Elymus glaucus* and *Sitanion jubatum*. Such a wide infection range for wheat mildew is in direct contrast to all previous records, since *Aegilops* embraces the only species outside the genus *Triticum* that have been reported infected by wheat mildew (10, 19).

Culture 2 does not differ from physiologic race 1 of Mains (10) on the basis of the following reactions of two differential wheat varieties: very susceptible—Malakoff C.I. 4898, and highly resistant—Axminster C.I. 1839. Mains reported the reactions of the largest number of grasses to wheat mildew prior to the present study, and, except for a few species of *Aegilops*,

TABLE 1.—*Summary of infection experiments with accessions of grasses and cereals tested to eight cultures of Erysiphe graminis*

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Aegilops crassa Boiss.	1[a]	1	....	2	....	....	....	....	2	....	....	2	....	....	....	....
A. cylindrica Host	1	....	....	1	1	....	....	....	....	1	....	1	....	1	....	....
A. triuncialis L.	1	....	....	1	....	....	....	....	....	....	....	1	....	....	....	....
Agropyron caninum (L.) Beauv.	....	1	....	1	....	....	....	1	....	1	....	....	....	....	....	....
A. cristatum (L.) Beauv.	....	4	....	4	3	....	....	4	1	3	....	4	3	1	....	1
A. desertorum (Fisch.) Schult.	....	1	....	1	1	....	....	1	1	....	1	....	....	1	....	1
A. inerme (Scribn. and Smith) Rydb.	1	7	....	12	12	....	....	11	11	....	10	1	9	1	....	4
A. intermedium (Host) Beauv.	....	1	1	....	1	....	....	1	1	....	....	....	....	....	....	1
A. repens (L.) Beauv.	....	1	....	1	2	....	1	1	1	....	1	....	1	....	....	1
A. semicostatum (Steud.) Nees	....	1	....	1	1	....	....	1	1	....	1	....	1	....	....	1
A. sibiricum (Willd.) Beauv.	....	5	....	5	5	....	....	5	4	1	2	3	2	3	....	....
A. smithii Rydb.	....	1	....	1	1	....	....	1	....	1	1	....	1	....	....	....
A. spicatum (Pursh) Scribn. and Smith	2	3	1	1	13	....	....	14	8	2	11	2	5	1	1	....
A. striatum (Steud.) Nees ex Hook.	1	....	1	....	1	....	....	1	....	....	....	....	....	....	....	....
A. subsecundum (Link) Hitchc.	....	1	....	1	1	....	....	1	1	11	....	....	1	....	....	....
A. trachycaulum (Link) Malte	....	14	1	13	13	....	1	13	3	....	6	8	4	7	....	3
Agrostis alba L.	....	4	....	4	....	4	....	4	....	4	....	4	....	4	....	4
A. exarata Trin.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
A. hiemalis (Walt.) B.S.P.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
A. interrupta L.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
A. palustris Huds.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
A. scabra Willd.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
A. spica-venti L.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
A. stolonifera L.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
Alopecurus aequalis Sobol.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
A. pratensis L.	....	....	....	....	....	3	....	3	....	3	....	3	....	3	....	1

[a] Numbers in table refer to the number of accessions tested of each grass species.

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Arrhenatherum elatius (L.) Mert. and Koch.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
Avena sativa L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
Beckmannia crucaeformis (L.) Host.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
B. syzigachne (Steud.) Fernald	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
Bromus arvensis L.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	....
B. brizaeformis Fisch. and Mey.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. carinatus Hook. and Arn.	....	....	....	....	....	2	....	2	....	2	....	2	....	2	....	2
B. catharticus Vahl	....	3	....	3	....	3	....	3	....	3	....	3	....	3	....	....
B. commutatus Schrad.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. inermis Leyss.	....	6	....	6	....	4	....	6	....	6	....	6	....	6	....	1
B. japonicus Thunb.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	....
B. macrostachys L.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	....
B. macrostachys var. *lanuginosus* Boiss.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. marginatus Nees	....	7	....	7	....	8	....	4	....	8	....	8	....	8	....	....
B. mollis L.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	....
B. polyanthus Scribn.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. purgans L.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	....
B. rigidus Roth.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. rubens L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. secalinus var. *velutinus* (Schrad.) Koch	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. squarrosus L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. tectorum L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. tectorum var. *glabratus* Spenner	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
B. vulgaris (Hook.) Shear	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
Dactylis glomerata L.	....	8	....	8	....	8	....	8	....	8	....	8	....	8	....	8
Deschampsia caespitosa (L.) Beauv.	....	2	....	2	....	2	....	2	....	2	....	2	....	2	....	2
D. danthonioides (Trin.) Munro	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
Elymus canadensis L.	1	2	....	6	4	1	....	5	1	4	2	3	1	4	....	2
E. canadensis var. *robustus* (Scribn. and Smith) Makenz. and Bush	....	1	....	1	1	....	....	....	1	....	1	....	....	....	....	....
E. condensatus Presl	1	1	....	4	4	....	....	4	4	....	4	....	3	....	....	1
E. dahuricus Turcz.	1	....	....	1	1	....	....	1	1	....	1	....	1	....	....	1
E. glaucus Buckl.	2	1	1	3	2	2	....	3	1	2	....	4	....	4	....	....
E. junceus Fisch.	1	....	....	1	1	....	....	1	1	....	1	....	1	....	....	....
E. sibiricus L.	1	....	....	1	1	....	....	1	1	....	1	....	1	....	....	....
E. triticoides Buckl.	1	....	....	1	1	....	....	1	....	....	....	1	....	1	....	1
E. villosus Muhl.	....	1	....	1	....	....	....	....	....	....	....	1	....	....	....	....
E. virginicus L.	....	4	....	4	3	....	....	4	....	4	1	2	2	1	....	....
E. virginicus var. *glabriflorus* (Vasey) Bush	....	1	....	1	1	....	....	1	....	1	....	1	....	1	....	....
E. virginicus var. *intermedius* (Vasey) Bush	....	1	....	1	....	1	....	....	....	1	....	1	....	....	....	....
Festuca arizonica Vasey	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
F. elatior L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
F. elatior var. *arundinacea* (Schrib.) Wimm.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
F. gigantea (L.) Vill.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
F. idahoensis Elmer	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	1
F. obtusa Spreng.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
F. occidentalis Hook.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
F. octoflora Walt.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
F. ovina L.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
F. rubra L.	....	1	....	1	....	2	....	2	....	2	....	2	....	2	....	....
F. rubra var. *commutata* Gaud.	....	1	....	1	....	1	....	....	....	1	....	1	....	1	....	....
F. thurberi Vasey	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
Holcus lanatus L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....
Hordeum bulbosum L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	....

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
H. gussoneanum Parl.	....	1	....	1	....	....	....	1	....	....	....	....	....	....	....	....
H. jubatum L.	....	1	....	1	....	....	....	1	....	....	....	....	....	....	....	....
H. jubatum var. *caespitosum* (Scribn.) Hitchc.	....	1	....	1	....	1	....	....	....	1	....	....	....	....	....	....
H. murinum L.	....	1	....	1	....	1	....	....	....	1	....	....	....	....	....	....
H. nodosum L.	....	1	....	1	....	....	....	1	....	1	....	1	....	....	....	....
H. vulgare L.	....	2	13	2	....	2	7	....	....	2	....	2	....	2	....	2
Hystrix patula Moench	....	....	....	....	1	....	....	1	1	....	....	1	....	1	....	1
Koeleria cristata (L.) Pers.	....	5	....	5	....	5	....	5	....	6	....	6	....	6	....	6
Lolium multiflorum Lam.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
L. perenne L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
Millium effusum L.	....	1	....	....	....	1	....	1	....	1	....	1	....	1	....	....
Phalaris arundinacea L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
Phleum pratense L.	....	3	....	3	....	3	....	3	....	3	....	3	....	3	....	3
Poa ampla Merr.	....	7	....	7	....	31	....	30	....	31	....	31	....	31	....	7
P. arachnifera Torr.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
P. arctica R. Br.	....	1	....	1	....	....	....	....	....	....	....	....	....	....	....	....
P. arida Vasey	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
P. bulbosa L.	....	1	....	1	....	4	....	4	....	4	....	4	....	4	....	1
P. canbyi (Scribn.) Piper	....	3	....	3	....	7	....	7	....	7	....	8	....	7	....	3
P. compressa L.	....	3	....	3	....	3	....	3	....	3	....	3	....	3	....	3
P. curta Rydb.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	....	1
P. cusickii Vasey	....	1	....	1	....	2	....	2	....	2	....	2	....	2	....	1
P. epilis Scribn.	....	1	....	1	....	2	....	2	....	2	....	2	....	2	....	1
P. gracillima Vasey	....	2	....	1	....	3	....	3	....	3	....	3	....	3	....	3
P. interior Rydb.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
P. juncifolia Scribn.	....	1	....	2	....	4	....	4	....	4	....	4	....	4	....	....
P. nemoralis L.	....	3	....	3	....	8	....	8	....	8	....	8	....	7	....	1

TABLE 1—(*Continued*)

Grass species	Cultures of *E. graminis*															
	2		3		6		7		10		11		13		18	
	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
P. nervosa (Hook.) Vasey	....	....	....	....	....	2	....	2	....	2	....	2	....	2	....	....
P. nevadensis Vasey	....	3	....	3	....	6	....	....	....	6	....	6	....	6	....	3
P. palustris L.	....	3	....	3	....	6	....	6	....	6	....	6	....	6	....	3
P. pratensis L.	....	14	....	14	....	15	....	15	....	15	....	15	....	15	....	11
P. scabrella (Thurb.) Benth.	....	1	....	1	....	3	....	3	....	3	....	3	....	3	....	1
P. secunda Presl	....	3	....	3	....	6	....	6	....	6	....	6	....	6	....	3
P. sphondyloides Trin.	....	2	....	3	....	5	....	5	....	5	....	5	....	5	....	3
Polypogon monspeliensis (L.) Desf.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	1
Puccinellia distans (L.) Parl.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	1
Secale cereale L.	....	1	....	1	....	1	....	1	....	1	....	1	....	1	1	....
Sitanion hystrix (Nutt.) J. G. Smith	1	....	....	1	1	....	....	1	1	....	....	1	1	....	....	1
S. jubatum J. G. Smith	1	....	....	1	1	....	....	1	1	....	....	....	....	1	....	1
Trisetum flavescens (L.) Beauv.	....	....	....	....	....	1	....	1	....	1	....	1	....	1	....	....
T. spicatum (L.) Richt.	....	1	....	....	....	1	....	1	....	1	....	1	....	1	....	....
Triticum aestivum L.	1	1	....	1	....	1	....	1	2	....	....	1	....	1	....	1

all the wild grasses were highly resistant. Some of the grasses tested by Mains were represented also in the present study, and all were highly resistant to culture 2. Thus, the results of culture 2 show that where a comparison is possible the results are comparable with those of Mains (10). Furthermore, with the exception of *Aegilops*, if those grasses susceptible to culture 2 had not been tested, the results would be, in fact, a confirmation of previous conclusions about narrow host range. Of course, it may be that the wheat mildew tested by the writer was different from that tested by all previous workers. However, since culture 2 did infect species of *Aegilops*, *Agropyron*, *Elymus*, and *Sitanion*, it is necessary to make adjustments of the previous concept of the pathogenic potentialities of wheat mildew. It also adds emphasis to the necessity of testing a large number of accessions of many grass species, if a better knowledge of pathogenicity is to be obtained.

Culture 3. On *Hordeum vulgare*, from Ann Arbor, Mich.

Culture 3 did not produce unusual results on barley varieties. Reactions of barley varieties were as follows: highly resistant—Arlington C.I. 702, Hanna C.I. 906; very resistant—Duplex C.I. 2433, Goldfoil C.I. 928, Heil's Hanna C.I. 682, 244 C.I. 1021; moderately resistant—Black Hulless C.I. 666, Common Chile C.I. 663, Lynch C.I. 919, Nepal C.I. 595; moderately susceptible—Coast C.I. 276, Oderbrucker C.I. 940, Peruvian C.I. 335; very susceptible—Horsford C.I. 877, and Malting C.I. 1129.

For the first time infection by barley mildew is reported on grass species outside the genus *Hordeum*. To culture 3 there developed a moderately susceptible reaction on *Agropyron intermedium* and *A. trachycaulum*, a moderately resistant reaction on *A. striatum*, and a very resistant reaction on *A. spicatum* and *Elymus glaucus*. These results, which record infection of species of *Agropyron* and *Elymus* by barley mildew, are in disagreement with all previous published records. Marchal (12) obtained no infection on any wild grass species except *Hordeum*, but his results included only one collection for each of the following species: *Agropyron caninum*, *A. giganteum*, *A. repens* and *Elymus arenarius*. If the few wild grass species that proved susceptible had not been tested, the remaining negative results would have confirmed the concept of previous workers that the specialized races of *Erysiphe graminis* were restricted in infection range to the species of a single genus. Apparently, insufficient collections of grass species were tested in previous studies.

The results obtained with culture 2 from wheat and culture 3 from barley show that these mildew cultures can infect wild grasses. Since some of the infected grasses exhibited susceptible and very susceptible reactions in the case of wheat mildew and moderately susceptible reactions in the case of barley mildew, the results suggest that these mildews might be discovered eventually on wild-grass species. The mixed cultures studied from *Agropyron repens* and *Elymus dahuricus* present such situations.

Mixed culture on *Agropyron repens*, from Yakima, Wash.

The inoculation results proved interesting, since in addition to infection of many grass species, 2 varieties of barley (Horsford C.I. 147 and Malting C.I. 326) showed a very susceptible reaction after inoculation with conidia from the bulk culture on *Agropyron repens*. The infection of these barley varieties by conidia from *A. repens* was verified in 3 additional tests under conditions that excluded the possibility of foreign contamination by employing the glass lamp-chimney technique. The mildew on barley was increased and designated culture 7. As will be explained in greater detail, culture 7 had a narrow host range on wild grasses and, besides barley, infected chiefly *Agropyron repens*, the original host. The other portion of the mixture, designated culture 6, had a wide host range, and many wild-grass accessions were available by which it could be separated from culture 7.

Culture 6. On *Agropyron repens*, from Yakima, Wash.

Culture 6, an *Agropyron* mildew isolated from a mixed culture on *A. repens*, as described above, proved to be the most aggressive mildew culture studied, both in number of grass collections infected and in severity of the attack. All collections of all species of *Agropyron* exhibited some degree of susceptibility to this culture. Furthermore, infection was produced on *Aegilops cylindrica, Elymus canadensis, E. condensatus, E. dahuricus, E. glaucus, E. junceus, E. canadensis* var. *robustus, E. sibiricus, E. triticoides, E. virginicus* var. *glabriflorus, Hystrix patula, Sitanion hystrix* and *S. jubatum*. Such a wide infection range greatly conflicts with the concept that *Agropyron* mildew infects only species of *Agropyron*, which was established by Marchal (12) and has not been contested heretofore. Culture 6 is perhaps different from all the other *Agropyron* mildews studied by previous workers. Marchal's studies contain only a few species of *Agropyron* and no species of *Aegilops, Elymus, Hystrix* or *Sitanion*. Since the work of Marchal (12) and Salmon (27) represents the only information available on *Erysiphe graminis* from *Agropyron*, it appears that the long-held ideas about pathogenicity were probably based on insufficient data.

Culture 7. On *Hordeum vulgare* by infection with conidia from *Agropyron repens* from Yakima, Wash.

This culture originated on *Agropyron repens*, but was multiplied on barley varieties. Reactions of differential barley varieties were as follows: very susceptible—Arlington C.I. 702, Goldfoil C.I. 928, Horsford C.I. 147, Malting C.I. 326, Peruvian C.I. 925; moderately resistant—Black Hulless C.I. 666 and Nepal C.I. 595.

The only wild grass exhibiting any appreciable susceptibility to culture 7 was the accession of *Agropyron repens* from which it originated. If it were not established by these experiments that culture 7 had its origin on *Agropyron repens* there would be no hesitancy in referring it to *Erysiphe graminis hordei*. Since culture 3 from *Hordeum vulare* infected certain

accessions of grass species in the genera *Agropyron* and *Elymus*, it seems logical that "barley mildew" should occur on such wild grasses under natural conditions. It could be interpreted that culture 7 represents a barley mildew occurring on *Agropyron repens*. Further evidence in support of this contention is the observation that there was a light infection of *Erysiphe graminis* on cultivated barley plants in the field adjacent to the plants of *Agropyron repens* on which culture 7 was originally collected. Culture 7 produced very striking results on barley varieties. The very susceptible reaction on the variety Arlington C.I. 702 distinguishes culture 7 from all described races of barley mildew in the United States. This discovery of a new race of *Erysiphe graminis* occurring on *Agropyron repens*, but capable of infecting barley, increases considerably the significance of the results showing that barley mildew can infect wild grasses under both natural and artificial conditions.

Mixed culture on *Elymus dahuricus*, from Ann Arbor, Mich.

A vigorous culture of *Erysiphe graminis* on *Elymus dahuricus* was brought into the greenhouse from the writer's grass-pathology nursery at the University of Michigan Botanical Garden. An inoculation of grasses and cereals provided unusual results. Infection was produced on both wheat and barley, as well as many species of *Aegilops*, *Agropyron*, *Elymus*, *Hystrix*, *Hordeum*, and *Sitanion*.

These results demonstrated that either there was a mixture of mildew races on *Elymus dahuricus*, or that there existed a race of *Erysiphe graminis* on *Elymus dahuricus* that could infect species of all these genera. Two isolates of this bulk culture were studied.

Culture 10. On *Triticum aestivum*, from Ann Arbor, Mich.

Culture 10 originated from infection on wheat (Malakoff C.I. 4898) by conidia from the mixed culture on *Elymus dahuricus*. The infected wheat plants were isolated in a separate greenhouse. This culture was maintained by transfers of conidia to fresh wheat seedlings at 2-week intervals.

Inoculation of grasses and cereals with culture 10 resulted in the infection of many grass species in the genera *Aegilops*, *Agropyron*, *Elymus*, *Hystrix*, *Sitanion*, and *Triticum* (Table 1). Reactions of differential wheat varieties were as follows: very susceptible—Malakoff C.I. 4898; moderately resistant—Axminster C.I. 1839. The reaction on the latter variety was confined to the first leaf sheath. Thus, culture 2 from wheat and culture 10, which infects wheat but came from *Elymus dahuricus*, infect many of the same grass species; but culture 10 infected many more collections of grass species than did culture 2.

Culture 11. On *Elymus dahuricus*, from Ann Arbor, Mich.

Culture 11 was isolated on *Agropyron desertorum*, which had been infected with conidia from the bulk culture on *Elymus dahuricus*. Only a

moderately resistant reaction on *Agropyron desertorum* resulted from inoculation with this culture. However, a few conidia were transferred to *Elymus dahuricus*, and the resulting infection provided a more vigorous culture with abundant sporulation for inoculation purposes.

From the results in table 1 it is seen that culture 11, an isolate from *Elymus dahuricus*, was capable of infecting only species of *Agropyron* and *Elymus*. *Agroypron* species were found to be more favorable for the development of culture 11 than were species of *Elymus*, both in number of species and degree of susceptibility. More species of *Agropyron*, however, were tested than of *Elymus*.

Culture 13. On *Elymus condensatus*, from Pullman, Wash.

This culture was started from dry leaf material sent by George W. Fischer, who obtained the inoculum from this grass growing in the Soil Conservation Service nursery at Pullman, Wash. Results of inoculations of grasses and cereals with this culture, multiplied on *Elymus condensatus*, are given in table 1. Culture 13 produced some degree of infection on nearly

TABLE 2.—*Summary of the reactions of 23 accessions of cereals and other grasses to 8 cultures of Erysiphe graminis*

Grass accessions	Cultures of *Erysiphe graminis* and source genus							
	Hordeum	*Triticum*	*Agropyron*	*Agropyron*	*Secale*	*Elymus*	*Elymus*	*Elymus*
	3	2	6	7	18	10	11	13
	Types of reaction							
Aegilops cylindrica 219[a]	0[b]	3–4	3	....[c]	.....	0	0	0
Agropyron cristatum 153	0	0	2+	0	0	2+	0	2–
Agropyron desertorum 151	0	0	3–4	0	0	3–	2–	0
Agropyron inerme 108	0	0	2+	0	0	3	4	3
Agropyron repens 150	0	0	3–4	0	0	2–	3–4	0
Agropyron repens 370	.....	.....	4	3	.....	.....	.....	.....
Agropyron sibiricum 142	0	0	2–3	0	0	2–	0	1+
Agropyron smithii 152	0	0	2–3	0	0	0	3–	2–
Agropyron spicatum 130	2–	3	4	0	0	3	4	2+
Agropyron trachycaulum 121	0	0	2–3	0	.....	0	1	0
Agropyron trachycaulum 134	3	0	2–3	0	0	3	2–	3–
Elymus canadensis 162	0	3	1–2	0	.....	3–	2+	2+
Elymus condensatus 164	0	0	2–3	0	0	3–	2–	3–
Elymus dahuricus 176	0	3	4	0	0	3	4	2–
Elymus glaucus 173	0	2–	0	0	.....	1+	0	0
Elymus junceus 168	0	4	4	0	.....	4	2–	2–
Elymus triticoides 174	0	3	3	0	0	0	0	0
Elymus virginicus 214	0	0	2–3	0	.....	0	2–	4
Hordeum vulgare (Malting C.I. 1129)	4	0	0	4	0	0	0	0
Hystrix patula 252	.....	.....	3–4	0	0	2–3	0	.....
Secale cereale 371	0	0	0	0	4	0	0	0
Sitanion hystrix 179	0	3	4	0	0	2–	0	1–
Triticum aestivum (Malakoff C.I. 4898)	0	4	0	0	0	4	0	0

[a] Accession numbers given to grass collections by the writer.
[b] For explanation of these types of reaction see under Materials and Methods.
[c] A blank indicates no test was made.

all species of *Agropyron* and *Elymus* tested, and a very resistant reaction developed on *Sitanion hystrix*. Cultures 11 and 13 from species of *Elymus* are quite comparable in several respects. They infect species of *Agropyron* and *Elymus*, and the conidia of the two cultures are somewhat smaller than those of other cultures infecting grasses in the tribe Hordeae. Cultures 11 and 13 show a similar brownish color of their mycelia not observed in the other cultures. They, however, can be easily distinguished by differences in reactions of several grass accessions (Table 2). These two *Elymus* mildews differ from each other, but are closely allied and distinct from all other cultures studied.

In table 2 are given significant reactions of certain grass accessions and cereal varieties to the 8 cultures.

DISCUSSION

The results of this investigation demonstrates that the infection range of no culture of *Erysiphe graminis* here reported is restricted to the species of one genus. *Erysiphe graminis* hitherto has been divided into specialized varieties, each considered as limited to species of a single grass genus. Only a few instances of infection of species in closely related genera have been noted. The infection of species of *Aegilops* with wheat mildew by Reed (18, 19) and Mains (10) was not stressed because of the close relationship of *Aegilops* to wheat. The infection of *Arrhenatherum elatius* with oat mildew by Marchal (12) was confirmed by Reed (19). Reed assigns no importance to this fact because *Arrhenatherum* is taxonomically closely allied to *Avena*. The other and even more extraordinary example is the infection of *Hordeum silvaticum* (*Elymus europaeus*) with wheat mildew reported by Salmon (28). On the basis of the results of the present study the importance of the few records of infection of species of more than one genus by a given culture of *Erysiphe graminis* as noted by previous investigators appears to have been dismissed too lightly.

The 8 cultures of *Erysiphe graminis* described in this study have been shown by infection experiments to be pathogenically distinct. The differentiations were possible on the basis of the type of reaction produced on a group of grasses and cereals.

On the basis of the old concept holding that specialized varieties of *Erysiphe graminis* were restricted to species of a single genus, one would be justified in concluding, for example, that all races of *E. graminis* on species of *Agropyron* would be *Erysiphe graminis agropyri*. The results of the present study demonstrate that this is not necessarily the case. It might be *Erysiphe graminis tritici*, *E. g. hordei*, *E. g. agropyri*, *E. g. elymi*, or a mixture of these "specialized varieties." All of the above named "varieties" also could occur separately or as a mixture on certain species of *Elymus*.

The possibility of hybridization between different pathogenic races during the initiation of the perithecial stage is presented, since races that

attack *Triticum, Hordeum, Agropyron* and *Elymus* can occur together on the same plant of certain species of *Agropyron* and *Elymus*. Furthermore, because wheat and barley mildews are shown to infect grass species, wild grasses must now be considered in the epiphytology of the disease on cereals as sources of primary infection and perennial stations for the fungus. These statements are given considerable emphasis by the fact that a culture that could be classed as a new race of *Erysiphe graminis hordei* was isolated from *Agropyron repens* collected at Yakima, Wash., which produced a very susceptible type of infection on the barley variety, Arlington C.I. 702. This barley variety had been resistant to all 7 races of *Erysiphe graminis hordei* described for the United States by Mains and Dietz (11) and Tidd (30).

None of the cultures reported in this study can fulfill the requirement that a given specialized variety infects species of only one genus. However, if the descriptions of the various specialized varieties were emended to include the new infection results, most of the cultures could be assigned to previously recognized varietal names. Thus, culture 3 would be referred to *Erysiphe graminis hordei,* culture 2 to *tritici,* culture 6 to *agropyri,* and culture 18 to *secalis.* What, then, about the nomenclature of culture 10, which is comparable to "wheat" mildew but was isolated from *Elymus dahuricus* and culture 7 which is comparable to "barley" mildew but isolated from *Agropyron repens?* Do these cultures represent "wheat" and "barley" mildews on *Elymus* and *Agropyron,* or *Elymus* and *Agropyron* mildews infecting wheat and barley, respectively, or are they races not to be identified specifically as either gross or cereal mildew "varieties"? Cultures 11 and 13 might be considered as two races of *Erysiphe graminis elymi,* which was proposed as a morphologic variety by Homma (3) based on slight differences in conidial measurements.

This type of classification would still tend to obscure the pertinent points of new information, namely, that none of these cultures is restricted to species of a single genus, that several cultures overlap in host ranges, and that it is possible for 2 or more races, comparable to varieties of previous workers, to occur together as a mixture on certain wild-grass host plants, thus presenting the opportunity for hybridization between races. The use of varietal names may continue to be useful despite the difficulties that accompany their application in the instances described. It does not seem advisable to attempt a reclassification of the varietal system until more information is available.

Wide differences occur between the host ranges of the various cultures. Culture 6 from *Agropyron repens* infected the largest number of grass collections and species, including at least some degree of infection on all collections of all species of *Agropyron.* The rest of the cultures provide a gradating series of degrees of specialization down to culture 18 from *Secale cereale,* which besides this species produced a very weak infection on *Agropyron spicatum.*

All cultures reported here were collected from grasses in the tribe Hordeae and produced infection only on species in this tribe.

This study also has demonstrated that different strains within grass species vary considerably in reaction to *Erysiphe graminis*. These differences are of importance in the selection of economic grasses for disease resistance. Some grasses were outstanding for their resistance, the most noteworthy being *Agropyron trachycaulum* (183, 184, 185),[4] *Elymus virginicus* var. *intermedius* (208), *E. canadensis* (163), *E. glaucus* (172), *Hordeum nodosum* (177), *Lolium multiflorum* (158) and *L. perenne* (157). It must be remembered that these two species of *Lolium* might be susceptible to a mildew race from *Lolium* or from other grasses as yet not studied, and the other "resistant" grasses may be susceptible to other races of *Erysiphe graminis*.

In the studies of powdery mildew of wheat and barley, genetically uniform agronomic varieties have been available as differentials to distinguish the different pathogenic races. Few genetically selected strains of wild grasses are available at present, and most grass-disease investigations must employ variable collections of grass species. This is exemplified by the great variation of reaction to powdery mildew of plants within certain grass collections used in this study.

SUMMARY

Three hundred and eighteen accessions of 108 species and 9 varieties of wild and cultivated grasses of the genera *Aegilops, Agropyron, Agrostis, Alopecurus, Arrhenatherum, Avena, Beckmannia, Bromus, Dactylis, Deschampsia, Elymus, Festuca, Holcus, Hordeum, Hystrix, Koeleria, Lolium, Millium, Phalaris, Phleum, Poa, Polypogon, Puccinellia, Sitanion,* and *Trisetum*, as well as 15 varieties of *Hordeum vulgare*, 1 of *Secale cereale*, 2 of *Triticum aestivum* and 1 of *Avena sativa* were studied in regard to their reaction to 8 cultures of *Erysiphe graminis*.

In contrast to the previous concept of restriction of infection to a single host genus all the cultures studied produced infection on species of two or more genera.

Powdery mildew from wheat and barley infected wild grass species. Cultures comparable to wheat and barley mildews were isolated from naturally infected plants of *Elymus dahuricus*.

A virulent culture isolated from *Agropyron repens* infected the previously resistant barley variety, Arlington C.I. 702 and could be classified as a new race of *Erysiphe graminis hordei*.

Differences in reaction of strains within grass species were demonstrated in nearly every instance where infection was produced on a grass species containing more than one accession, and in a few instances differences in reactions of individual plants were found within accessions, thus affording an opportunity for selection for resistance. Several accessions of economic grasses have shown notable resistance to all of the cultures.

[4] Accession numbers given to grass collections by the writer.

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HOST–PARASITE RELATIONSHIP BETWEEN THE OAT PLANT (AVENA SPP.) AND CROWN RUST (PUCCINIA CORONATA)

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INTRODUCTION

Since 1705, when Tournefort (34), before the Académie Royale des Sciences, described parasitic molds as the cause of plant diseases and discriminated between those molds that obtain nourishment from living entities and those that thrive by inducing decay of inanimate materials, the problem of the relationship between host and parasite has been the object of no little study and speculation. We shall briefly recount the more important efforts to resolve that problem, a subject that can now be considered as a whole in the light of convergent cytological and cytochemical techniques.

REVIEW OF LITERATURE

In 1886, de Bary (6) published his classical paper on the parasitic behavior of *Sclerotinia libertiana* Fckl. He proved to his own satisfaction, at least, the presence of an active entity in the extracts of parasitized tissues of certain host plants subjected experimentally to the fungus. This entity or principle he found capable of inducing the disease effects he described. When he boiled the extracts containing this principle he succeeded in destroying its potency; whence his conclusion that the effect produced by this agent on the host-cell walls under its impact was none other than that of an enzyme.

We next find de Bary's deductions essentially confirmed by Marshall Ward (35), who, in a notable paper read before the Royal Society in London, concluded by way of summary that "all the evidence points to the existence, in the cells of the fungus, of enzymes or toxins, or both, and in the cells of the host-plant of antitoxins or similar substances, as the decisive factors in infection or immunity." He is frank to admit, however, that up to that time he had failed to isolate any such bodies.

Again, the facts as disclosed by de Bary were substantiated by Brown (9), who prepared from the germ tubes of *Botrytis cinerea* Auct. a markedly active extract. This he found capable of attacking and decomposing a variety of vegetable and fruit tissues and equally active in its effect on the leaves and floral organs of many plants, and the stems and leaves of numerous succulents. Heating at 60–70° C. effectively destroyed the extract. The author failed to separate the enzymic from the toxic principle, though he tried in various ways to do so.

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Stakman (29), after comparing the sequence of events in the infection of two varieties of *Triticum*, the one (Minn. 163) susceptible, the other (Khapli) resistant to *Puccinia graminis tritici* Eriks. and Henn., concluded that resistance to the rust is due to physiological incompatibility between host and parasite, a question calling for an answer through biochemical investigation. Later, the same author (30) states that the hypersensitiveness of the host seems to be common to plants that are somewhat resistant to *P. graminis tritici* and to those that are nearly immune from this rust. This hypersensitiveness becomes manifest very soon after fungal invasion of the host cells, and in some instances is visible even before such invasion occurs.

Reed and Crabill (25) observed in their study of cedar rust of the apple that an occasional rust-infection court died out at the center. They were of the opinion that this condition could be explained on the assumption that a deleterious product of the fungus diffused into cells adjacent to the invading hyphae and killed them before the fungus actually reached them. The rust organism, being an obligate parasite, is unable to grow in these dead cells; accordingly, its further progress is stopped. Thus, a seemingly paradoxical situation arises here. That is to say, the hypersusceptibility of the host is responsible for the death of the parasite.

In her study of infection phenomena resulting from invasion of Kanred wheat by a weakly virulent physiologic race of *Puccinia graminis tritici*, Allen (2, 3) observed that the cytoplasmic envelope of a growing haustorium of the invading mycelium disappears rapidly. This she interprets as a possible yielding by the fungus of some substance into the host cell. She observed that this contact between host and parasite was accompanied by a chemical change, but was unable to determine whether such change was due to the "mere physical presence of the haustorium or to some substance diffusing from it." Any attempt to disclose the basic facts underlying the true nature of rust resistance by commonly accepted cytologic technique cannot prove otherwise than unsatisfactory. It is too easy to mistake mere artifact for the actual and characteristic. Any interpretation of what one may, therefore, observe as having taken place in fixed and stained infected or other tissue as compared with the intravitally stained tissue cannot be other than an approximation of the truth.

Rice (27), in her studies on the nature of rust parasitism, concludes that the intrusion of the haustorium induces "offensive action" by the host nucleus. Again, she refers to Allen's (1) description of the relationship of the fungus to its resistant host as a "host-nuclear offensive," which, as she interprets it, is indicative of a chemotactic response to substances that diffuse out into the host cell from the haustorium. The reaction manifested by the invading fungus she interprets as induced by secretion from the host cells. Sappin-Trouffy (28) is of the opinion that through contact of the fungus haustorium and the host nucleus the invading parasite takes up certain products of host-cell metabolism. As to what these products are or how they become available to the parasite he does not say.

Pady (24), in his consideration of infection in *Gymnoconia interstitialis* (Schl.) Lagh., calls attention to the amount of mycelium within the leaf tissues of *Rubus* prior to formation of haustoria, and infers that some nourishment must derive directly to the fungus from the host cells with which the hyphae of the invading organism are in intimate contact. Thus does he add the weight of his opinion to that of other students of host-parasite relationship; but we are, up to this point, busy with spade work necessary to the disclosure of the means and *modus operandi* necessary to the establishment and maintenance of such relationship.

Ward (36) was the first to demonstrate experimentally the dependence of plant rusts on carbohydrates or on certain intermediate products of photosynthesis elaborated by their hosts. He proved, for example, that the mycelium of *Puccinia glumarum* (Schm.) Eriks. and Henn. would succumb to starvation in wheat leaves kept in an atmosphere void of carbon dioxide.

Mains (22) demonstrated experimentally the relation of light and carbohydrates to cell respiration of certain rust-infected plants. In his investigation of the carbohydrate relationship he found that in the absence of either light or carbon dioxide the growth of *Puccinia coronata* Corda or *P. sorghi* Schw. was retarded or stopped altogether because of dearth of carbohydrates. When he supplied these in the form of sugar solutions, *P. sorghi* resumed growth, even in the dark, on corn seedlings deprived of their endosperm.

A year later Dufrenoy (14a and b) published his observation on the accelerated transpiration and fatty degeneration of tissues affected by rust.

Arthur *et al.* (4) incline to the opinion that the rust fungus gains access to the host cell through a minute pore in the cell wall effected through an enzyme brought to bear on the cell wall at a point of contact between the haustorium mother cell and the host cell. They refer, too, to the "delicate balance" that apparently is established between the rust organism, on the one hand, and the host cells of a susceptible variety or species, on the other, with a consequent increase in size of the host nuclei of attacked cells and an accelerated metabolism in such cells. Rust-resistant plants show no such balanced behavior. Biochemically considered, as we shall presently see, they cannot.

Certain contributors to our knowledge of rust-infection phenomena, notably Beauverie (7) and, more recently, Thatcher (31, 32), have emphasized the evident influence of an active parasite on the "antibiotic struggle" that characterizes the host-parasite relationship.

Beauverie (7) is convinced that the destructive action of the parasite brings about a perturbed osmosis. The fungus, having penetrated the host tissue, takes up water, and the more so when its fructification is externally apparent, a stage of fungal development marked by a heightened respiration. All this results in a concentration of the cell sap and a dehydration of the cytoplasm. He states further that the fungus may exert an indirect

action, *i.e.*, secrete some diastases, under whose influence such large-molecule substances as starch, albumins, etc., are split into smaller molecules, more numerous, and crystalloid. There follows a marked increase in osmotic pressure.

Thatcher (32) demonstrated that in the germ tubes and haustoria of *Uromyces fabae* (Pers.) de Bary on *Pisum sativum, U. caryophyllinus* (Schrank) Wint. on *Dianthus caryophyllus, Botrytis,* and *Sclerotinia sclerotiorum* (Lib.) Mass., the osmotic pressure registered by the fungus was greater than that of the host, thus confirming MacDougal's (16) observation of the same phenomenon in his study of induced parasitism. Thatcher proved by means of permeability measurements of the tissue of rust-infected and noninfected plants that rust infection is attended by increased permeability of the plasmatic membrane of the host cells. This increased permeability he is inclined to attribute to some secretion from the rust fungus. On this observation Thatcher has based his hypothesis that "even an obligate parasite causes certain substances to leach from the host cells in its vicinity." It is his opinion that this hypothesis permits the extended survival of parasitized cells commonly observed among plant rusts. He suggests further (32) that the greater osmotic pressure of the parasite enables it to acquire water from contiguous host cells and that increased permeability makes available to the parasite those cell solutes to which the plasma membrane is no longer semipermeable. The author states further that this change in permeability is not the only physiological modification of the host cell essential to rust nutrition. He does not, however, suggest that dextrose is the source of the carbon utilized by the rust fungi.

More recently, Thatcher (33) states that his observation of living sections of rust-infected tissue over a period of several years has convinced him that extracellular rust enzymes may have a pertinent relationship to such differences in host response as characterize the mesothetic reaction. In several instances he observed extracellular fatty materials, which he interpreted as a sequel to rust invasion. Also, from time to time he had noted evidence of changes in the hydrophily of the host protoplasm. These several observations, "considered in relation to the universal secretion of enzymes by parasitic fungi and to the supposed nature of the plasmatic membrane," led him to conclude that there probably is some truth in the suggestion that the "rust fungus secretes at least two enzymes," one a protease and the other an enzyme capable of splitting lecithins from lecithoproteins, in short a "lipase."

MATERIALS AND METHODS

The investigation here considered was confined to crown rust (*Puccinia coronata*), cultured in field and greenhouse at Louisiana State University in 1942 and 1943. Inoculations in the greenhouse were made with urediospores of physiologic race 1, obtained from Beltsville, Maryland. Because

of their several types of specific response to infection by this physiologic race, young plants of the following oat varieties were inoculated on November 4, 1942, under nearly identical conditions: Bond, Victoria, Rainbow, Markton, Richland, and Bond × D69. Similarly, seedling plants of these same varieties were inoculated in December, 1942, and in January and February, 1943.

Tangential freehand sections of host tissue from immediately beneath the rust sorus were immersed in saline or sucrose solutions containing 0.1 per cent neutral red for vital staining. Other and similar sections were immersed in a saturated solution of 2–6 dichloroquinone imide in a 0.2 per cent solution of sodium barbiturate buffered at pH 8.6. Still other sections were immersed in a 0.01 per cent solution of para-phenylenediamine chlorohydrate, and some in a molybdenum reagent (H_2SO_4 20.8 cc., diluted by adding distilled water to 250 cc.) in which was dissolved 6.41 g. of ammonium molybdate. To 5 cc. of this was added, at time of application, 1 cc. of the following: 5.75 g. of sodium bisulphite in 85 cc. of distilled water + 0.5 g. 1-amino-2-napthol-4-sulfonic acid + 5 cc. of a 20 per cent solution of sodium sulphite.

The convergent use of these techniques gave a good picture of the comparative behavior of the phenolic compounds, of the polyphenol oxidases (as evidenced in decompensated respiration), and of the distribution and relative dispersal of the phosphorus compounds in the resistant or susceptible varieties of oats.

Of the reagents employed, the molybdenum proved most valuable, as the blue phospho-molybdenum compound forms almost instantaneously where phosphorus is free and more slowly where the phosphorus is more firmly bound into nucleoproteins, phosphorylated sugars, and lipoproteins. Thus, by means of the microscope, a proper idea of the dispersal of such compounds in rusted tissues was obtainable from sections of host material that had been immersed for stated periods of time ranging from a few seconds to several hours.

This same reagent also affords a picture of the oxidizable polyphenols in the tissues—one that conforms to the pictures obtained through the use of the para-phenylenediamine or the chloroimide reagents. Here, again, in all cases, the expression of the definitive coloration at the site of oxidizable phenols required from a few minutes to hours or even a few days. It was found advantageous, when sections had to be examined repeatedly for days, to preserve them in the refrigerator between periods of examination. The stock solutions of the reagents also were similarly stored.

CYTOLOGICAL CONSIDERATIONS BEARING ON THE VACUOLAR SYSTEM OF THE HOST-PLANT CELL

The response of the host-plant cell infected by a rust fungus may vary all the way from abrupt structural changes, resulting in sudden death, such as characterizes hypersensitivity flecks, to a progressive evolution of cell constituents, giving the host cell and its parasite the mutual benefit of a

long survival. When the leaf tissues contiguous to the infection court remain green, while the rest of the leaf becomes yellow, the infected cells even may enjoy a prolonged life.

Those two extremes of host response and all intermediate aspects can now be interpreted cytochemically in the light of recent knowledge of the interrelationship between the respiratory systems of the living cell and its cytological structure.

The ability of a parasite to thrive in the tissue of its host has been correlated (20, 21, 32) with its higher osmotic pressure: the 24 per cent solution of sucrose, which plasmolyzes the cells of the oat plant proximate to the court of infection, does not plasmolyze the hyphae of *Puccinia coronata* nor the host cells that lie beyond the court of infection.

This plasmolysis of affected cells, followed by deplasmolysis on prolonged immersion in the sucrose solution, has been interpreted as evidence of "increased permeability." But the vacuolar solution in the cells about the court of infection, where plasmolysis may be observed, shows other changes besides those ascribable to reduced osmotic pressure: 1. The vacuolar solution in these cells tends to become dispersed into numerous vacuoles differing not only in shape but also in the nature of their vacuolar solution. This dispersal of the vacuolar solution was recently considered by Reed and Dufrenoy (19) in that part of their paper devoted to cell secretion. 2. Coacervation, according to Dobry (13), intervenes as a limiting factor, so affecting the osmotic pressure as to render it almost nil. Coacervates of differing composition may coexist within the cell without any interfering osmotic phenomena. Through coacervation, cells may store substances in the liquid state where they remain available for metabolism, although they are as effectively protected, physicochemically, as though they were in a solid state. The vacuolar compounds that most directly pertain here are monophenols such as pyridoxin (vitamin B_6), diphenols (catechol), and oxidation-polymerization products. Normally, there is present in the vacuolar solution of the guard cells of the stomata and in that of the intervening epidermal cells of leaves of the oat varieties Victoria, Bond, etc., a compound that stains an intense blue with 2–6 dichloroquinone imide. In the other cells the compound may be too dilute to give a perceptible color reaction within the vacuolar solution, but becomes perceptible when coacervated.

Plasmolysis does not actually afford a way of measuring "osmotic pressure" in the vacuolar solution, as the osmotic pressure computed from the freezing point of expressed sap may be significantly lower than that calculated from plasmolysis. There exist within the living cell, besides the physical forces that play as osmotic pressure, some forces pumping water into the vacuole, from outside the cell, in the same way that there are forces active in the absorption of solutes. These forces involve an expenditure of energy. That energy is furnished by respiration—more precisely, by that specific part of respiration linked up with the cell structure and dependent upon the integrity of that structure. Commoner and Thimann (12) empha-

sized the interrelationship of the auxin action and cell respiration in the utilization of malic acid during growth of the coleoptile of oat seedlings, but present no data to account for the forces concerned in cell expansion. Berger and Avery (8), on the other hand, found in the coleoptile a number of the components of the Szent-Györgyi-Krebs respiration phosphorylation cycle, among which malic dehydrogenase was prominent.

The mechanism concerned in the transfer of energy from the oxidative to the assimilatory process appears to be a phosphate cycle with storage of the energy as phosphate-bond energy, and its release by subsequent splitting of the phosphate esters (23).

The phosphorus linkages operate in dehydrogenase systems whose activity is seemingly dependent on the cytological structure of the cell. The decline or "phospholyse" of the phosphorylated cytological constituents becomes evident in "fatty degeneration" of and the advent of myelin figures in the cells (Fig. 2, *ap*).

Following the course of one-half the glucose passing through this cycle, five "energy-rich phosphate bonds" may be derived, two on the fermentation pathway of the glucose passing from glucose to pyruvate; two as acetylphosphates, with oxidation of the two alpha-keto acids (pyruvic and keto glutaric); one with Kalckar's enol-phosphate, found on oxidation of fumaric or malic acid (15). Therefore, the transfer of one mole of triose through the cycle involves about one energy-rich phosphate bond for each of the six primary dehydrogenations.

Respiratory processes in the cell involve a series of phenomena: (a) phosphorylations; (b) oxidations or dehydrogenations of the phosphorylated metabolites; (c) synthesis of glucids from the products of the dehydrogenations, at the expense of the energy thereby liberated ("Pasteur reaction") (15).

Synthesis of glucids evidencing the Pasteur reaction may explain how concentration of sucrose may increase in parasitized tissues, or how starch becomes synthesized where it would not normally occur, or it may account for an increase in osmotic pressure.

From the time of Lavoisier the fundamental question as to how atmospheric oxygen, at relatively low temperature, acquires the property of performing the work of partial and complete oxidation has claimed the attention of both physiologists and chemists.

The experimental and theoretical study of catalytic auto-oxidation brought to the fore the "activation" of molecular oxygen, either (a) by addition to a nonsaturated system, or (b) by the activating influence of a metallic catalyst.

The first possibility involves oxidases or substances of nonsaturated character that absorb molecular oxygen and, in part, utilize it in the oxidation of other materials in the process of metabolism.

Simultaneously, hydrogen peroxide is produced by the hydrolysis of those primary oxides, and, by means of peroxidases, is activated for the purpose of oxidation.

Oxidases perform, not as catalysts, but as oxidizing enzymes, substances that undergo chemical change. For instance, we cannot dissociate "polyphenol oxidases" from the polyphenols while being oxidized (dehydrogenated) to quinoids.

The second possibility involves metals that can exist either as *ous* or *ic* compounds.

Iron, long ago (1705), was detected in the ash of plant tissues. Almost simultaneously it was found in chemical solution in plant tissues; but it took two centuries for iron to be recognized as the active group of cytochrome oxidases or of hydrogenases.

Hydrogenase is an iron compound active in the ferrous, but inactive in the ferric form, and behaving as the cytochrome-like "Pasteur enzyme" (17).

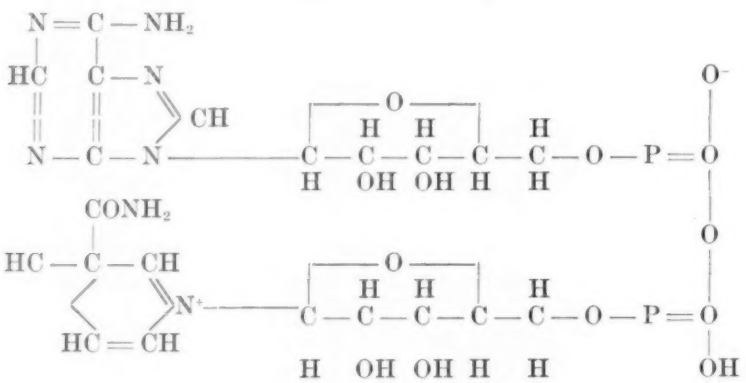
There are two ways of blocking the oxidations in the cell: either by replacing competitively the oxidizable metabolite by an unoxidizable compound (such as KCN), or by replacing O_2 by a nonoxidizing agent (such as CO).

As to hydrogenase activity, the importance of the phosphorus relationship is twofold, *i.e.*, in the phosphorylation of the coenzyme and in the phosphorylation of the hydrogen donor.

Dehydrogenases are dehydrogenating enzymes, activating hydrogen in phosphorylated glycerol or glyceric acid. The action of dehydrogenases is followed by that of the co-dehydrogenases, which transfer hydrogen. Following the discovery of these agents, it became clear that oxygen uptake is, to a marked degree, independent of CO_2 formation; hence, we can conceive of compensated *vs.* decompensated respiration, not in terms of O_2/CO_2 balance but in terms of reversible *vs.* irreversible dehydrogenations of metabolites.

The need of a coenzyme for the lactic, malic, and beta-hydroxybutyric dehydrogenases has been known since the systematic study of their properties.

Following is the structural formula for coenzyme I, diphosphopyridine nucleotide. (See Ann. Rev. Bioch. **10**: 230. 1941.)



Those dehydrogenases needing a coenzyme for their activation are inhibited by nucleotidases, which liberate inorganic phosphorus from the phosphorus linkages.

Examination of those substances that are oxidized in the cell discloses the fact that, in general, they are such as undergo the chemical changes involving loss of hydrogen (*e.g.,* in dehydrogenation) concomitantly with the building up or breaking down of energy-rich phosphorus linkage.

Phosphorus is the biochemical element *par excellence.* "Phosphorus mirabilis," as soon as it was discovered, attracted the attention of Huygens in France, of Boyle in England, of Leibnitz in Germany. Since then its biochemical prominence has become progressively evident, and in the study of our present problem we shall focus our discussion on phosphorus linkages.

Phosphorus Metabolism

The rust, as an obligate parasite, draws on the host cell for all of the materials it metabolizes after its reserves within the urediospore have been exhausted by the germ tube, appressorium, and stomatal vesicle. The cell contents of the urediospore was, prior to germination, rich in phosphorus that was strongly bound and capable of remobilization on germination of the spore. When this original supply of reserve material is exhausted and the phosphorus is again bound into energy-rich linkages within the germ tube, the appressorium, and stomatal vesicle, all of the phosphorus needed by the growing hyphae must be derived from the host.

The comparative cytochemical study of rust on a resistant and a susceptible host discloses a direct correlation between the ability of the rust fungus to produce an extensive mycelium and sporulate, on the one hand, and its ability, on the other hand, to draw upon a wider expanse of host tissue for phosphorus.

A normal cell may be assumed to be permeable to $PO_4^{\equiv}$ or to the salts of $PO_4^{\equiv}$ with cations, such as K^+, but impermeable to large molecules wherein PO_4 would be tied up by pentose phosphorylation to proteins, as in nucleotides. Tissues stained with the molybdenum reagent bring out the fact that the cells affected by rust and surviving infection release phosphorus compounds. In this respect they behave like cells that have been irradiated by ultra-violet radiations at sublethal doses (19) and that release phosphorus compounds acting as growth-promoting substances for neighboring cells. It may, therefore, be inferred that surviving infected cells release to the intercellular spaces phosphorus compounds that can be remetabolized by the rust hyphae, but that, also, inasmuch as they are not recaptured by the hyphae, may induce some reactions in the cells around the court of infection.

Therefore, gradients of phosphorus concentrations would become established not only between the infected host cells and the rust hyphae, but, as a whole, between the tissues of the court of infection and those immediately surrounding. There would result an influx of available phosphorus around

the actual court of infection into those tissues that face impending invasion by the encroaching hyphae, contrary to what would happen in healthy tissue.

Phosphorus is likely to be translocated there from the more remote tissues, mostly in the form of phosphorylated sugars. As the phosphorus thereby made available to the growing hyphae is being consumed the residue would be deposited in the form of glucose, sucrose, or starch; therefore accounting for that seemingly paradoxical result reported by Allen (1) in the case of mildew on wheat leaves, where high carbohydrate content and high respiration are intimately connected with growth of the parasite. That correlation would seem to be a general one and to be necessary in the case of all obligate parasites, rusts as well as mildews.

NONCOMPENSATED RESPIRATION

High respiration in infected tissues is concomitant with noncompensated respiration, and noncompensated respiration is concomitant with the disturbance of cell structure; noncompensated respiration means that O_2 absorption is increased without CO_2 emission being correspondingly increased, as part of the O_2 is used up for the dehydrogenation of the phenolic compounds to quinones, with formation of H_2O. The ultimate result of the interaction between rust and infected cells or between those infected cells within the infection court and those beyond that specific area will depend upon the severity of the decompensation of respiration, which, itself, will depend upon the dispersion of the dehydrogenases.

A disturbance of the energy-rich phosphorus linkages, as the cell faces infection, will result in a severe decompensation of respiration: phenolic compounds in the vacuolar solution, being no longer protected from irreversible dehydrogenation, form quinones, which diffuse out of the vacuolar solution into the cytoplasm, which they "fix" (26), and thus give rise to the hypersensitivity flecks (Fig. 1). The cells in those flecks are killed so quickly that they have no chance to induce metabolic responses in the nearby surviving cells.

Quite different is the case when noncompensated respiration allows the concomitant survival of infected cells and infecting hyphae. Here, such respiration is not made evident by the dehydrogenation of the phenolic compounds to brown derivatives and by the discoloration of the tissues, as in hypersensitivity flecks; but it can be demonstrated by immersing freehand sections of infected tissue in such reagents as para-phenylenediamine, 2.6 dichloroimide . . . , which give bright-colored quinoid derivatives on being dehydrogenated by the noncompensated activity of the oxidases.

Vital staining with those para or ortho compounds yielding bright-colored quinoid derivatives on dehydrogenation demonstrates that there is widespread in plant tissues an enzyme corresponding to what was known originally as tyrosinase. This enzyme catalyzes not only the aerobic oxidation of polyhydric phenols, where the –OH groups are in either the para or ortho position, and the oxidation of the amines, where the –NH groups

are in either the para or the ortho position, but, in the presence of catechol, also catalyzes the aerobic oxidation of certain monohydric phenols.

Whether tyrosinase, as here defined, represents a specific enzyme or a group of enzymes among which such specific enzymes as polyphenol oxidases or catechol oxidases should be discriminated, has been the subject of much recent controversy. What we are here concerned with in contributing to our understanding of the host-parasite relationship are these facts, *i.e.*, that the aerobic oxidation of various ortho-dehydroxy phenolic compounds is catalyzed at widely different rates by tyrosinase and that the oxidation

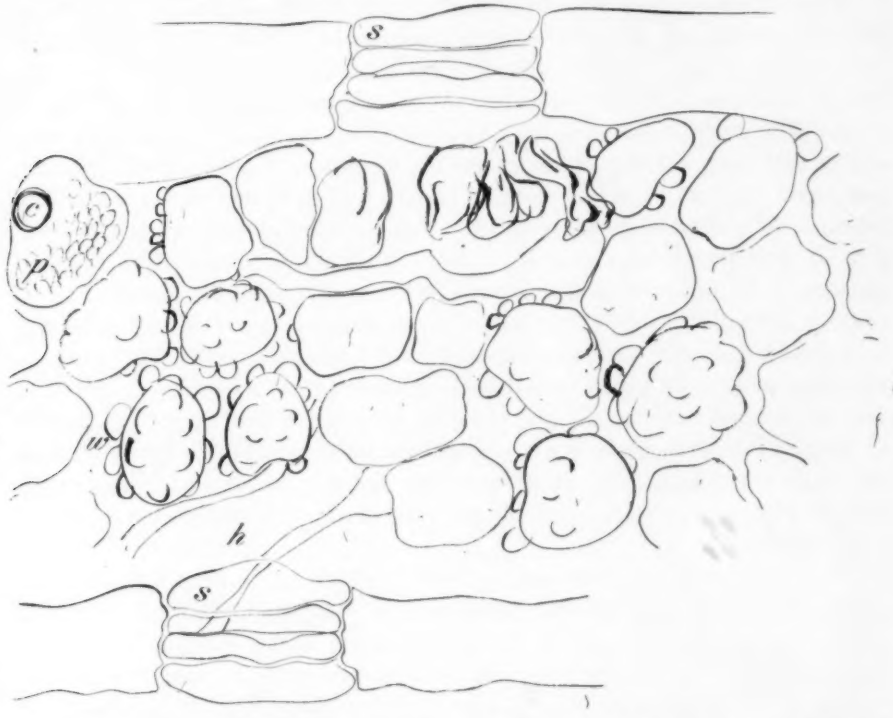


FIG. 1. Tangential section of rust-infected leaf of the variety Victoria. Inoculated November 4; examined November 10 in a solution of 2–6 dichloro-quinone imide. The penetration of the hyphae (*h*) through the stomata resulted in the collapse of the cells immediately adjoining, as evidenced by the folding in of the cell walls. The cells farther away show extrusions homologous to those featured by R. Allen as "pectic warts" (Jour. Agr. Res. [U.S.] **34**: (8), pl. 3, fig. C, pp. 702–703. 1927).

products of these compounds vary in their tendency to inactivate the tyrosinase.

For instance, Baker and Nelson (5), in 1943, point out that catechol is oxidized very rapidly to quinones, as evidenced by the rapid browning of the reaction mixtures. They assume that these quinones exert an inhibitory effect on some intermediate link in the respiratory system of the living cells. Conversely, protocatechuic acid (4-carboxycatechol) is oxidized more slowly, yielding quinone at such a slow rate that the rest of the respiratory system

is able to reduce the quinone as soon as it is formed, thereby enabling the acid to serve as a hydrogen carrier. Baker and Nelson obtained their results from a study of the respiration of living cells of potato-tuber tissue. Slices of the tuber were subjected to a Warburg respirometer, which enabled them to record the amount of oxygen intake and CO_2 outgo, at a temperature of 25° C. Experimental evidence thus obtained supports their conclusion that at least 85 per cent of the oxygen uptake of such respiring tissue enters the chemistry of the potato cell by way of a tyrosinase-catalyzed oxidation.

From the foregoing investigations we may derive the following concept, which bears directly upon the general problem of host-parasite relationship, *i.e.*, that there exist in plant tissues, concomitantly, various phenolic substances that can be dehydrogenated by an enzyme system of the tyrosinase type. The dehydrogenation may be reversible, and those substances then act as hydrogen carriers as long as the respiration is not severely decompensated. Indeed, a slight decompensation, corresponding to some disturbance of the cytological structure, entailing increased permeability, may step up the dehydrogenation of monophenols to polyphenols and of polyphenols to quinones with correlative increase of O_2 intake that enhanced respiratory activity, which corresponds to a feverish condition of the tissues, is observed whenever cells survive attack by a given parasite; but they survive because they can keep up a dehydrogenase system whereby the quinones can be reverted back to the polyphenols; in other words, they will survive as long as they can retain a certain specific structural relationship between the phosphorus linkages and the cell constituents, as well as preserve the integrity of some –SH groups linked up with the nucleoproteins acting as dehydrogenases.

Wherever the quinones fail to be rehydrogenated, they will catalyze further oxidations, inhibit some links in the respiratory systems, and bring about death of the cells and formation of brown necrotic areas in the rust-infected leaf. The interrelationship between host and parasite as evidenced by resistance or susceptibility seems, therefore, largely dependent on the rate of dehydrogenation of polyphenols to quinones. Where this rate is not so rapid as to outstrip the rate of reversible reaction, the cell or cells can survive; both host and parasite can more or less compatibly live together; the host is susceptible. If the rate of quinone production is too rapid, the cells are killed before the rust fungus can get anything from them (Figs. 1 and 2). Thus no continuing parasitic relationship can be established, and the host is resistant. In this way decompensated respiration can be detected wherever hyphae of the rust grow and appropriate unto themselves phosphorus compounds from the host cells. A gradient of phosphorus concentration between the hyphae and the infected cells is demonstrated by staining with the molybdenum reagent. If the cells become too seriously depleted in phosphorus, they become the site of phenolic dehydrogenation to quinones, their cytoplasm becomes fixed by the quinones and they die. If, however,

they survive, they exhibit, concurrently with decompensated respiration, definite changes in structure, involving mostly the cytoplasmic interphases. These changes may be properly described as secretion phenomena: The central vacuole, originally present in the normal cell, may secrete a number of secondary vacuoles; the vacuolar contents, the colloids of which are chiefly

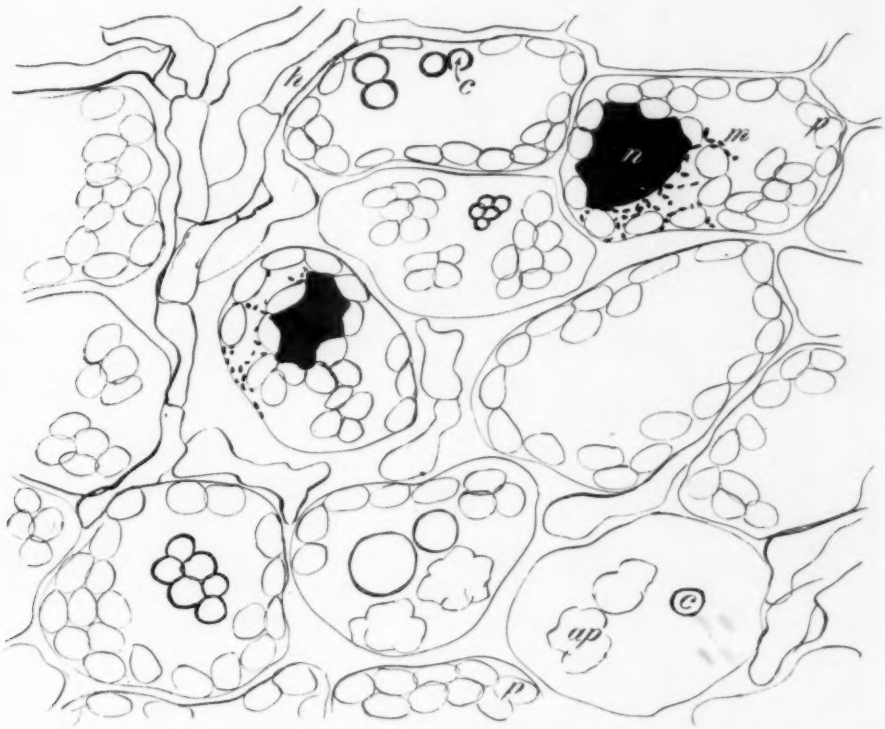


FIG. 2. Tangential section of rust-infected leaf of the variety Victoria below sorus of *Puccinia coronata*. Infected December 30, 1942; examined January 28, 1943; section treated with the molybdenum reagent. In two of the cells the nucleus (*n*) stained a deep blue, as well as neighboring mitochondria (*m*) (featured solid black). Intercellular hyphae (*h*) show no material staining blue with the molybdenum reagent, indicating that whatever phosphorus had been available has been translocated to the few urediospores formed (not shown). The chloroplasts (*p*) (merely outlined) remain appressed against the cell wall in some cells, and their distribution outlines the central vacuole, wherein the phenolic compounds have become coacervated into a number of spherical bodies, sharply outlined by the limiting membrane (*c*). In other cells the chloroplasts became agglutinated (*ap*), eventually losing their identity and fusing into a "myelin body."

represented by phenolic compounds, may not be apportioned equally to all of those vacuoles.

Cell Secretion

Normally, the volume of the oat-leaf cell is occupied for the most part by the vacuolar solution, rich in colloids, represented chiefly by phenolic compounds. That vacuolar solution stains a deep purple or violet with neutral red, occupies a vacuole in the central part of the cell, and is surrounded by the cytoplasm. The cytoplasmic vacuolar interphase is the site

of surface-tension phenomena, allowing for the irregular shape of the vacuole.

As the cell is affected by the parasite, the vacuolar solution becomes dispersed into several vacuoles. This dispersal may be considered as representing a phenomenon of external secretion (as contrasted with internal

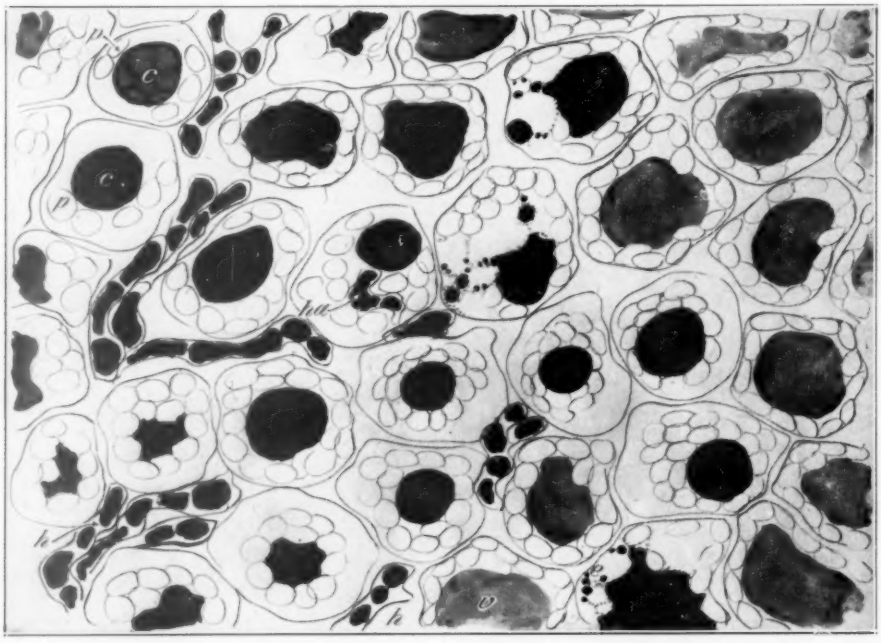


FIG. 3. Tangential section of rust-infected leaf of the variety Markton below sorus of *Puccinia coronata*. Inoculated December 30, 1942; examined February 17, 1943. Vital staining with neutral red in 24 per cent sucrose solution. Vacuolar material staining purple to orange, featured black or grey. The vacuoles in the intercellular hyphae (*h*) contain several small vacuolar precipitates, shown in solid black. In the vicinity of the hyphae the cells of the chlorenchyma show plasmolysis in the 24 per cent sucrose solution; the chloroplasts (seen in outline only) become agglomerated, as the cytoplasm retracts from the cell wall, around the contracting vacuole. Where the cell plasmolyzes, the vacuolar solution stains orange or red, as contrasted to pink or purple in the normal cells, indicating a shifting of the pH from the normal 5.5 towards 7. In many cells in the vicinity of the hyphae, the phenolic compounds, and particularly the pyridoxin from the vacuolar solution, became coacervated into a spherical mass (*c*), sharply outlined by deeply staining limiting boundary. As the vacuolar solution around the coacervate undergoes plasmolysis, the cytoplasm, carrying the chloroplasts, may become tightly appressed against the coacervate. In some cells, the vacuolar solution shows no coacervation; instead, it evidences an excretion or a secretion of materials to the outside, as the vacuole becomes irregularly lobed and buds out peripheral vacuoles sharply outlined by a row of lipid droplets in the enmeshing cytoplasmic strands. Farther away from the hyphae, the cells retain their turgor in the 24 per cent sucrose solution, the vacuolar solution (*v*), stains pink to purple, and occupies the huge central vacuole pressing the cytoplasm and plastids against the cell wall.

secretion, presented under the heading "coacervation") (Fig. 3). The original vacuole may form "buds," which become detached as so many secondary vacuoles. Conversely, these secondary vacuoles may lack in colloidal material. At the interphase, with interfacial surface tension, each vacu-

ole tends to become perfectly spherical. Neutral red stains the contents of these secondary vacuoles orange red, thus demonstrating a shift of pH from normal acidity (approx. pH 5.5) toward neutrality. These smaller spherical vacuoles are the site of highly noncompensated oxidase activity, and give a bright-color response for quinoids on treatment of the tissues with para-phenylenediamine or 2–6 dichloro-quinone imide.

Concomitant with that secretion of materials from the mother vacuole into the newly formed smaller peripheral vacuoles, in the same cell, there occurs a secretion of intracellular material into the intercellular spaces correlatively with what has been discussed as "increased permeability."

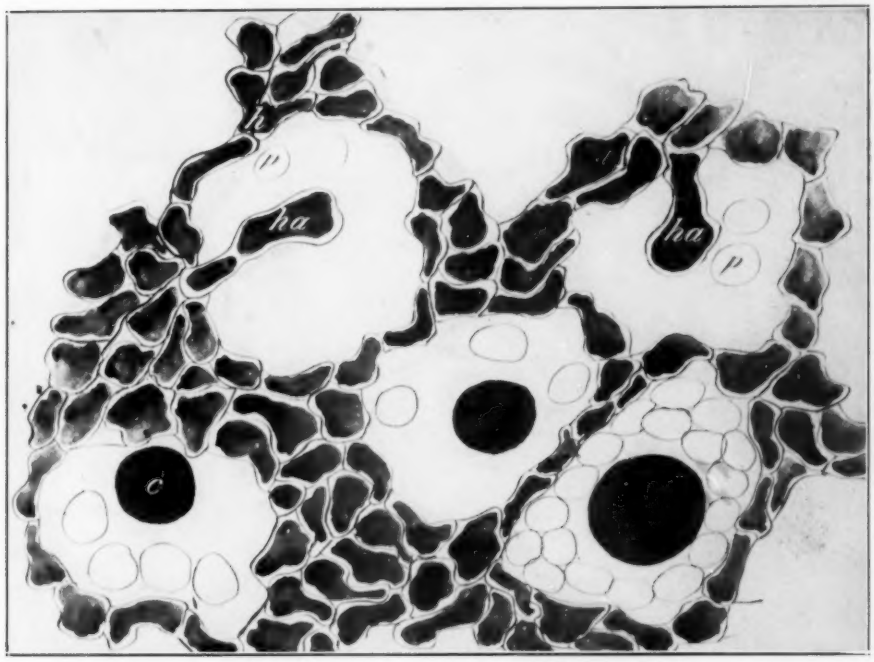


FIG. 4. Tangential section of rust-infected leaf of the variety Rainbow below sorus. Inoculated December 30; examined January 28 in a solution of neutral red in 24 per cent sucrose. The cells of the chlorenchyma are enmeshed in the pseudo-tissue of the intercellular hyphae (*h*), comprising cells each of which contains a large red-staining vacuole (featured grey). The two upper chlorenchyma cells contain each an haustorium (*ha*) filled with the vacuolar solution occupying one or two vacuoles. The lower cells contain each a coacervate (*c*) with a deeply staining limiting layer surrounding a lighter stained core (featured solid black), (*p*) chloroplasts, in outline.

Coacervation

The most conspicuous type of secretion occurs within the vacuolar solution itself through that particular process described by Bungenberg de Jong (10) as "coacervation." The colloidal materials, originally uniformly distributed throughout the vacuolar system, may become unequally apportioned to various drops therefrom, as the central vacuole secretes smaller vacuoles at its periphery, as described above.

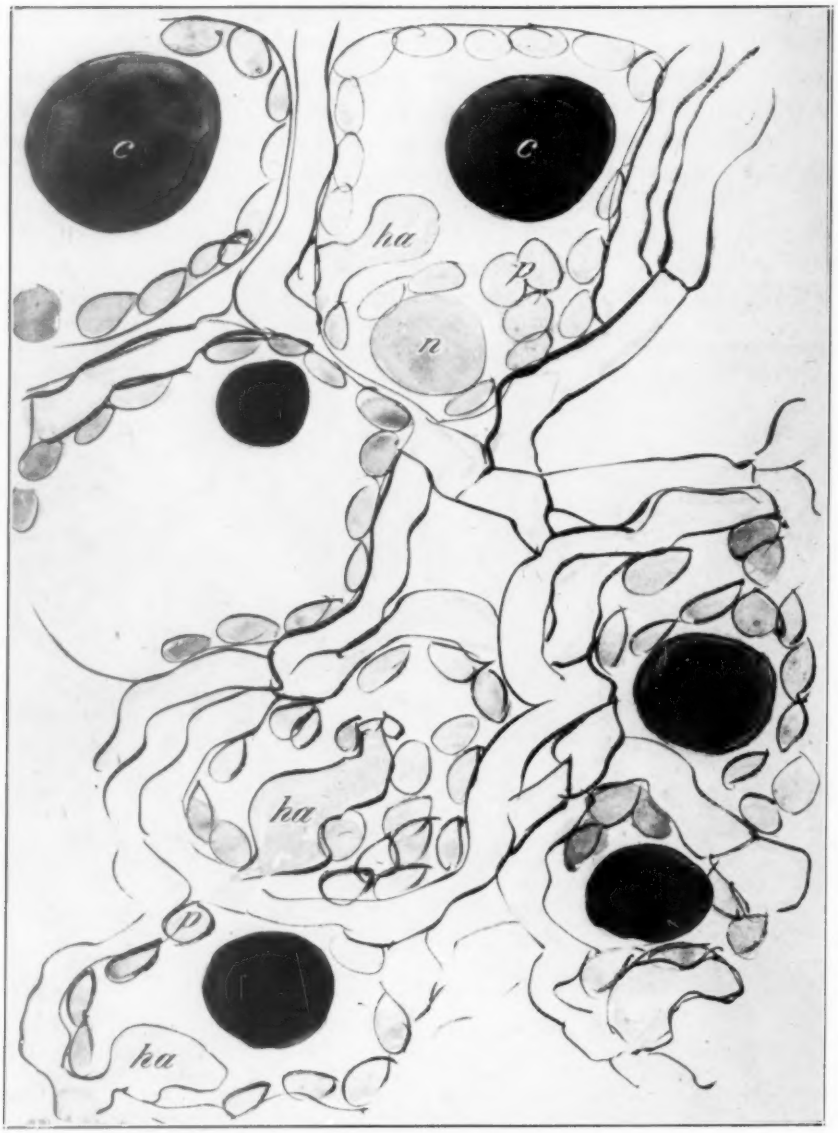


FIG. 5. Bond inoculated with crown rust from Bond January 14; examined February 14, 1943. Freehand tangential section below sorus, through patch of green tissues surrounding the sorus, from a leaf that had turned wholly yellow. After treatment for 4 days in the molybdenum reagent at 5° C. the nuclei (*n*) showed deep blue, as phospho-molybdenum blue had formed from the phosphorus compounds. The chloroplasts had retained their green color, showing that the chromo-proteins in these plastids were still intact, and did not release the compounded phosphorus. The large spherical coacervates (*c*) appeared deep brown red as the result of the oxidation of the phenolic compounds therein through the agency of the molybdenum reagent. Haustoria in cells (*ha*) showed a faint blue color due to phospho-molybdenum blue; the intercellular hyphae (*h*) are almost void, showing only a faint bluish coloration of the thin cytoplasm; it, therefore, appears that whatever phosphorus compounds had been obtained from the host had been translocated to the few urediospores formed (not featured in the figure). This section shows the coacervates as entirely different and independent from either the haustoria or the nuclei.

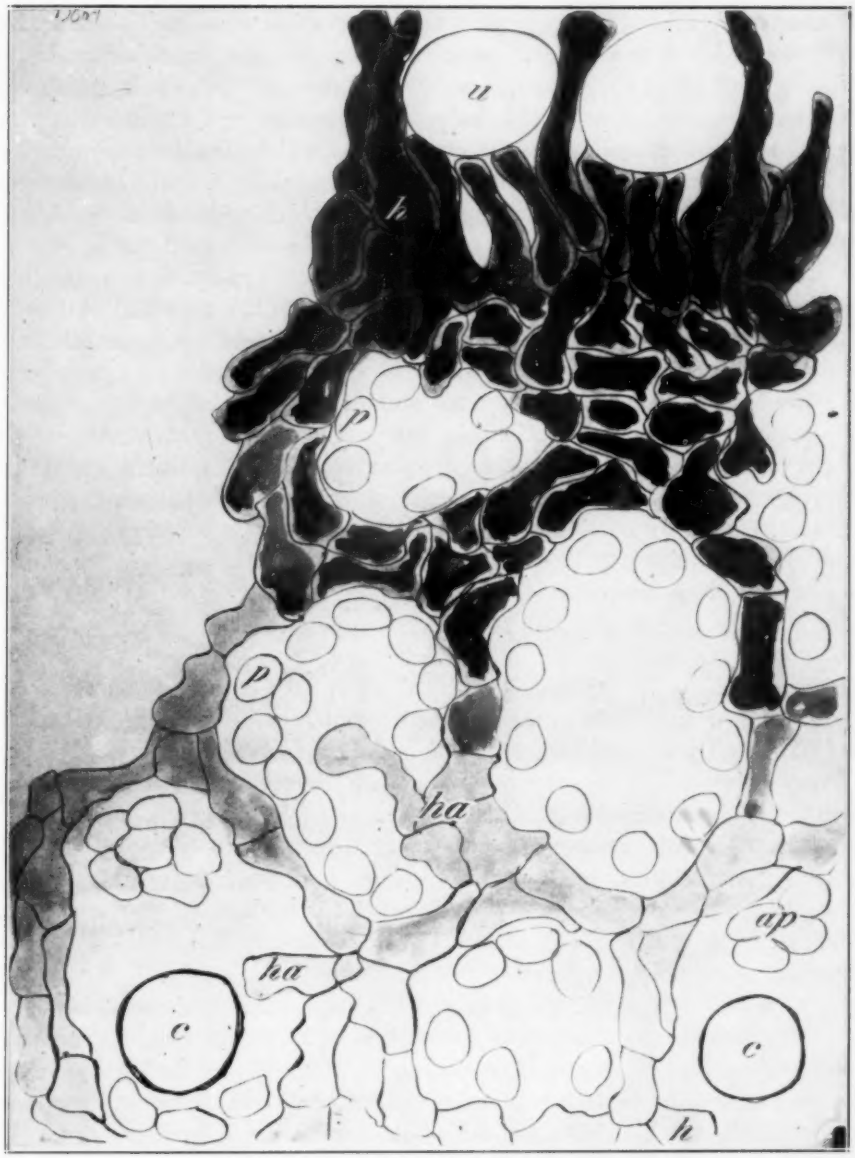


FIG. 6. Bond, inoculated January 14 with urediospores of race 1 of *Puccinia coronata* collected from Bond; examined February 5 in the molybdenum reagent. The hyphae (*h*) showed a gradient of intensity of development of the blue phospho-molybdenum reagent, as featured by the intensity of grey. The two urediospores (*u*) at the top showed their natural orange-red due to the carotenoids (the phosphorus in the urediospores is firmly bound with cell constituents and is unmasked only after several days in the reagent). Each of the two cells at base contains a large coacervate (*c*), sharply outlined by its phosphatid envelope and each containing the coacervated phenolic compounds, which the oxidizing effect of the molybdenum reagent has stained a deep red (*p*). Chloroplasts in outline. The chloroplasts are represented by their outline only; they were retaining their natural green color; the phosphorus is so firmly bound in the nucleo-protein constituent of the chromo-protein that it can be unmasked only after several days in the reagent. Some chloroplasts (*ap*) become agglutinated. The nucleus in each cell has not been featured; the nucleo-protein in the nuclei does not bind the phosphorus so firmly as one finds it in the plastids, and the nuclear phosphorus would give a blue color after 24 hours' treatment in the reagent. Note again that coacervates (*c*) are entirely independent from haustoria (*ha*).

Conversely, through the process of coacervation, these colloids may become apportioned to a special inclusion within the vacuolar solution, in the vacuole itself (Fig. 4). Then, if a freehand section of tissue in which colloids have become coacervated is immersed in neutral red or any other vital dye, the stain accumulates in the coacervates, which then become manifest as bright-colored spherical bodies, each surrounded by a well-defined membrane and suspended in the vacuolar solution, which no longer contains any stainable colloid, and, therefore, comes out of the bath unstained.

Coacervates, such as occur in crown-rust-infected leaves of the oat plant, belong to what Bungenberg de Jong and Kruyt (11) classified as "autocomplex coacervates," where the central core of coacervated phenolic compounds is permanently separated from the vacuolar solution by a membrane of phosphatids. Such coacervates are stable; they can best be demonstrated by immersing freehand sections in the molybdenum reagent, where the coacervated phenolic compounds will be oxidized to red quinoids, while the phosphorus from the phosphatid membrane will yield phospho-molybdenum blue (13), sharply outlining the contour of the coacervate itself (Figs. 5 and 6). This blue can be deepened to black by subsequent treatment in 1 per cent aqueous haematoxylin. The section can then be dehydrated rapidly with absolute alcohol and mounted in euperal.

CONCLUSIONS

Establishment of the parasitic relationship between the leaf cells of the oat plant, on the one hand, and the invading hyphae of the rust fungus (*Puccinia coronata*), on the other, depends on the release of phosphorus compounds by the host cells, which thus make available to the parasite their phosphorus. This release may be—very probably is—correlated to increased permeability, but also depends on a marked degradation of the phosphorylated compounds, a process referred to by Haag (16) as "phospholyse." This attempted physical interpretation of a cytological phenomenon does, however, leave us still lacking necessary enlightenment as to the facts basic to our understanding of the host-parasite relationship.

Cytochemical examination of the affected tissues reveals changes that can best be described as secretion phenomena. These appear to be homologous to the phenomena in either plant or animal cells following any stimulus, physical, chemical, or pathological, such as might be induced by a parasite or other pathogenic agent.

In all cases the affected cell gives up to the surrounding medium some phosphorus compounds, which it would normally retain intact. In the cells of an oat plant affected by rust there occurs, concomitantly with the excretion of materials into the intercellular spaces, an internal secretion within the vacuolar solution itself—a secretion resulting in the coacervation of phenolic compounds, mostly pyridoxin.

Indophenol-blue-forming phenolic compounds, such as pyridoxin, are widespread in the vacuolar solution of cells of the oat plant, and most

abundant in the vacuoles of the guard cells and of the long epidermal cells in line with the stomata.

Coacervation of those phenolic compounds seems correlated with the dispersion of the nucleotides or phosphoproteids in the cell and to the resulting decompensation of respiration. The establishment of the host-parasite relationship entails a decompensation of respiration that may still permit the host cell to survive. If, on the other hand, the decompensation be so severe as to prove rapidly lethal, the rust fungus no longer behaves as a parasite but as a pathogen, inducing necrotic spots characteristic of hypersusceptibility.

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THE MECHANISM OF WILTING CAUSED BY FUSARIUM BULBIGENUM VAR. LYCOPERSICI[1]

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The first important hypothesis on the nature of vascular wilts was formulated by Atkinson (3) in 1892. His investigations on cotton wilt demonstrated that the pathogen is a vascular parasite; and Atkinson postulated that the plants wilted because the fungus utilized the nutrients present in the tracheal fluid and thereby starved the host. This hypothesis was soon superseded by the plugging theory, which assumed that the wefts of mycelium observed in the vessels of the host caused wilting by hindering the movement of water to the upper parts of the plant (12, 34, 35, 48, 49, 54). Most of the recent investigators, however, report the very rare occurrence of mycelial wefts, and that the hyphae usually grow sparsely along the inner walls of the tracheae (1, 2, 6, 10, 19, 20). Even if occlusion did occur in some of the vessels there seemed to be sufficient lateral diffusion to keep the plant in a turgid condition (8, 11). Other types of occlusion, such as the formation of tyloses or gums in the xylem (4, 22, 23, 24, 37, 47), also have been suggested, but these may be secondary factors and not the primary cause of wilting. Similar materials also have been observed in bacterial wilts (27, 53). Another explanation for wilting in flax has been advanced by Tochinai (52), who suggested that *Fusarium lini* might produce enough carbon dioxide to form a gas pocket in the tracheae and thereby break the transpiration stream. Link (32) and Mann (33) ascribed the wilting of potatoes to an infection and necrosis of the root tissues, which limited the water-absorbing powers of the plant; but Tisdale (51) and Grossman (19) pointed out that, in flax, such necrosis takes place only after the plant is already dead. Vascular fungi may even cause wilting by destroying the mesophyll tissues of the leaves. According to van der Meer (53), van der Lek observed that *Fusarium tracheiphila* grew out of the vessels and attacked the mesophyll of the leaves of cucumber. Only after these tissues were attacked did the plant wilt.

The inadequacy of the foregoing explanations of wilting led to the development of the toxin[2] theory, now most generally accepted. It is based, for the most part, on the fact that toxins are produced by the different vascular fungi, when they are grown on synthetic nutrient media, and on the ability of these toxins to wilt excised shoots (2, 6, 9, 15, 19, 21, 30, 56). Vascular discoloration, which is a diagnostic character for these diseases, also has been

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[2] In this paper the term toxin is used to indicate a substance produced by a living organism that is deleterious to a plant.

attributed to these fungi. Analysis of the toxic filtrates from cultures of vascular parasites has yielded conflicting results, and the toxic activity has been variously attributed to different components such as nitrates, enzymes, acids, and alcohols (4, 14, 15, 20, 21, 42, 43, 44, 57). The more recent studies of Schaffnit and Lüdtke (46), Ahmet (1), Grossman (19), and Elpidina (13) indicate that the toxic fractions of the fungal filtrates may be amines. The toxin theory has been strengthened by the recent studies of Gottlieb (18) on fusarial tomato wilt. He reported the presence of toxins in the tracheal systems of wilted plants and their absence in the fluids obtained from noninoculated turgid plants.

There have been many theories to explain the action of the toxins on the host plant. Van der Lek, according to Rudolph (44), attributes the discoloration of the vessels to the corrosive action of a toxin, while Hursh (25) points out a correlation between the browning and an inactivation of the toxin. On the other hand, Picado (41), who also believed that the discoloration is due to metabolic products of the fungus, distinguished between discoloring action and the wilting effect. Pethybridge (40) believed this discoloration due to two components: the innate color of the fungal hyphae and another factor that is independent of the parasite.

A more important problem is the role of the toxin in disturbing the water relations of the plant. Bewley (4) and Higgins (22) postulate the secretion of substances that act upon the living cells, these cells, in turn, producing the gums that are the direct cause of wilting. Zentmeyer (60), in contrast, considered the tyloses and gums of secondary importance to the direct action of the toxins in causing wilting. Another vital question still unsatisfactorily answered is the kind of tissue the toxin affects. Hursh (26) thought the toxins acted on the vascular tissue, where they were deposited or inactivated. When stems were placed in fungus-culture filtrates, some of the cells at the exposed surface collapsed, thereby interfering with normal transpiration. If the ends of these stems were then cut off and the seedlings placed in water, the plants recovered. According to Hursh, permanent wilting sets in only when considerable functional tissue has been destroyed. Supporting this interpretation are the observations recently made by Maclean and Walker (34). In wilts of potatoes caused by *Fusarium avenaceum*, *Fusarium solani*, and *Fusarium oxysporum*, they observed that some of the small xylem vessels had collapsed and interpreted the collapse as indicating a dissolving action by the fungus.

Various investigators have suggested that the toxin might act on the leaf. As early as 1921, Young and Bennet (58, 59) believed that the toxin acted on the cell membranes, rendering them less permeable. Thus, under conditions of high transpiration, not enough water could reach the tissues to enable them to retain their turgidity. A similar hypothesis was presented by Clayton (8), who stated that either an increase or decrease in permeability could account for the wilting. Pertinent data on this subject have been presented by Linford (31), in transpiration studies on pea wilt,

from which he postulated increased permeability in diseased tissues. Fisher (16) also suggested that wilting of tomatoes was due to permeability changes in the cell membranes. Thatcher (50) measured the permeability of plants that wilted in fungal filtrates, and found that an increase in permeability was correlated with the wilted condition. More direct evidence that the toxin acts on the leaf tissues in some plants has been presented by Dawson (11) in his work on the *Cephalosporium* wilt of daisies. The most prominent symptom of this disease prior to actual wilting is an intense yellowing of the leaves. Dawson was able to reproduce this condition in the cells of the leaf by detaching them from the plant and placing them in a filtrate from the culture of the parasite. The mesophyll and palisade cells became an intense yellow and the chlorophyll was disorganized. Ahmet (1) believed that the plant could adjust itself to the new water relations and remain turgid if the pathogen was not too virulent, or other conditions did not favor an acute wilt. An adjustment of this type, he postulated, would account for the ability of some infected plants to continue functioning for some time, as occurs in the chronic wilts.

The object of the present investigation was to ascertain the immediate cause and mechanism of wilting of tomatoes due to *Fusarium bulbigenum* var. *lycopersici* (Bruschi) Wr. and R. Four principal phases of the problem were studied: the extent and effect of plugging; the production of toxins by vascular pathogens and other fungi; the presence of toxins in the tracheae of infected plants; and the effect of such toxins on the physiology of the host.

EXPERIMENTAL STUDIES

Effect and Extent of Plugging

Although the plugging theory of wilting has been discarded by most of the current pathologists, there is little direct evidence that it plays no part. More conclusive evidence on this subject was sought by a histological study of the growth and development of the pathogen in the different plant parts and by a study of the movement of water through infected and noninfected stems.

Bonny Best tomato plants were inoculated with *Fusarium bulbigenum* var. *lycopersici* by the Wellman procedure (55), and wilting occurred about 2 weeks later. Noninoculated plants were used as checks. Sections of tissue from the roots, 1 inch below the ground line, and from the stem at 1 inch and 9 inches above the ground and 1 inch below the uppermost petiole, were plated out on potato-dextrose agar. Fusarium cultures were isolated from the roots of 61 of the 63 inoculated plants, from 63 of the stem sections that were cut 1 inch above the ground line, and from 39 of the stem sections cut 9 inches above the ground line. No Fusarium species were ever isolated from the region of the uppermost petiole, indicating that any action of the fungus to cause wilting must begin at a distance from the leaf, the site of actual wilting. Twenty-one of the isolates from the roots and stems were

tested for pathogenicity, and all of them caused wilting in the inoculated tomato plants. From similar tissue sections of the check plants only 2 colonies of *Alternaria* were obtained that were considered as contaminants and unrelated to the disease.

At the time of wilting, half-inch sections of each of the above mentioned tissues were washed and fixed in formic acid, acetic acid, and alcohol solu-

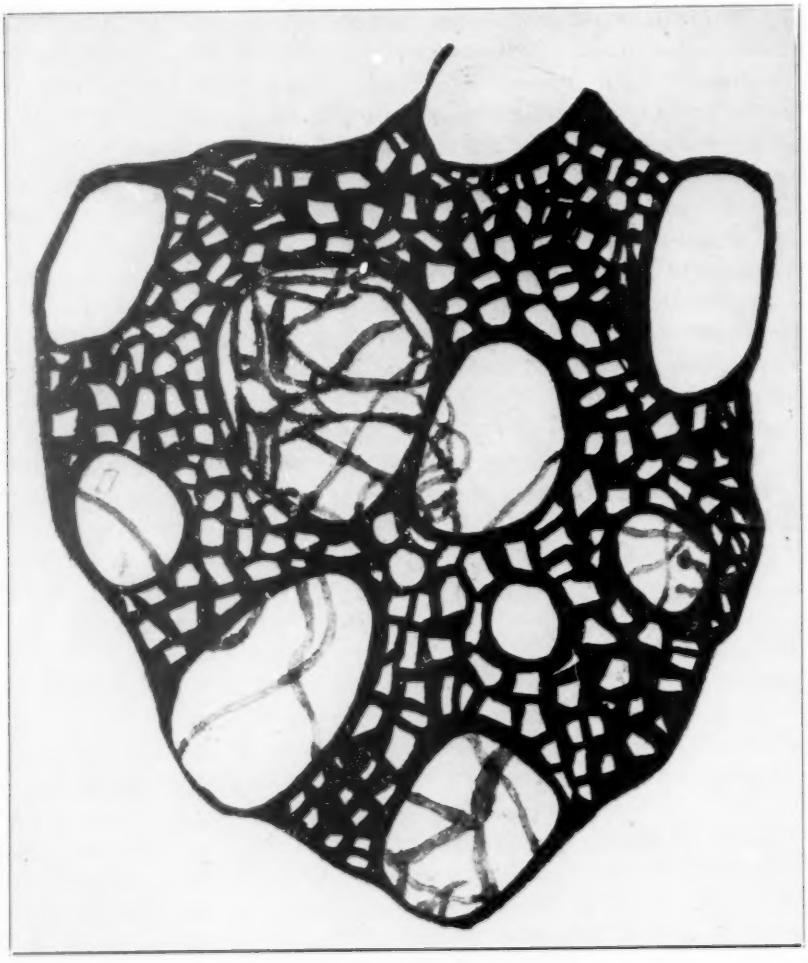

FIG. 1. Camera-lucida drawing of cross section of vascular bundle in lower portion of tomato stem infected with *Fusarium bulbigenum* var. *lycopersici*.

tion. Hand sections were made and the vascular tissues were studied microscopically. These studies confirmed the results of the culture studies, for all of the inoculated plants had mycelium in the lumen of the larger vessels of the roots and in the two lower portions of the stem, but none near the top of the shoot. No mycelium was present in any portion of the check plants. Approximately 45 per cent of the large vessels in any stem section contained

mycelium, although in some sections this ranged as high as 61 per cent. No plugging was observed in any of the sections made 9 inches above the soil line. But in the lower stem and in the roots, some of the vessels were well plugged with a mass of anastomosing and criss-crossing hyphae, occupying almost the entire cross-sectional area of the vessel (Fig. 1). Many of the

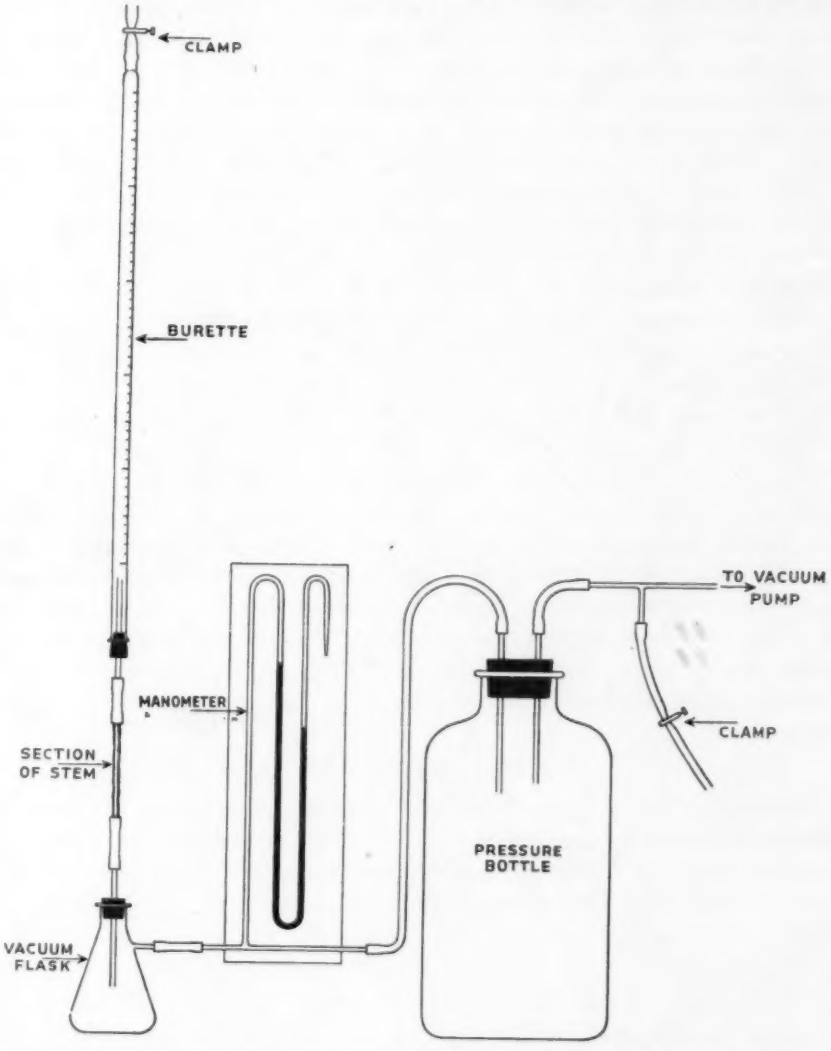

FIG. 2. Flowmeter for measuring the movement of fluids through stems.

vessels, however, contained only a few hyphae that formed a loose weft in the lumen. When one considers the length of tracheae that might thus be filled, it is apparent that the xylem could become a very inefficient transport organ. Nevertheless, even if the occluded vessels become nonfunctional, the remaining ones might still transport sufficient water to maintain turgidity of the plant.

Because many of the vessels were plugged, attempts were made to measure the movement of liquids through infected and noninfected stems. The movement of water through Fusarium-infected stems should be hindered if the obstruction by mycelium were responsible for wilting.

A flowmeter, therefore, was designed to measure the rate of flow of water through a section of stem (Fig. 2). An inverted burette containing water was connected to a section of the stem, which had been cut under water, by a short length of glass tube; and the lower end of the glass tube was then connected to another glass tube leading into the vacuum flask. The flask in turn was connected by suitable tubing to a mercury manometer and vacuum pump. A large bottle between the pump and the flask acted as a reservoir to cushion any changes in pressure, and an extra outlet from the T tube was used to regulate the pressure. Any length of stem could be accommodated by raising or lowering the clamp that held the burette. The stems were covered with petrolatum and a pressure of 13 cm. less than atmospheric was applied to the stem. This gave a flow of about 0.5 ml. of water in 30 min. through a 15-cm. section. Repeated trials demonstrated the precision of the apparatus. For example, 5 trials on a stem section of a noninoculated plant gave the following results: 1st run, 0.48 ml.; 2nd run, 0.46 ml.; 3rd run, 0.49 ml.; 4th run, 0.47 ml.; 5th run, 0.47 ml.

Upon further investigation it was discovered that the procedure could not be used for diseased tomatoes. The lower portions of the stems usually were hollow because of the disintegration of the pith, a condition that seemed more severe in infected than in normal plants. Thus, results would not be comparable even when stems of the same size were used. Although the upper portion of the stem was still solid, it could not be used because it contained very little mycelium. Therefore, no conclusions could be drawn as to the effect of plugging on the movement of water through diseased stems.

TOXINS IN CULTURE

The production of toxins in culture by vascular parasites has been the principal evidence supporting the toxin theory of wilting. But such evidence has not been considered entirely conclusive, since the metabolism of microorganisms may vary with the culture medium (5). Rosen (43), for example, showed that the ability of toxins from *Fusarium vasinfectum* to cause wilting in cotton plants depends on the culture medium on which the fungus has been grown. Lathrope (28) thought that the aldehydes in the filtrates of cultures of *Fusarium cubense* were responsible for wilting in the host, but he was unable to find such compounds in the diseased plants. Various fungi other than vascular parasites also produce toxins. It was, therefore, interesting to test a large variety of fungi and determine their ability to produce wilt toxins.

Twenty-six fungi including vascular parasites, root-rotting organisms, leaf-spotting fungi, and saprophytes (Table 1) were studied. All the fungi were grown in Richards' solution, except *Ustilago zeae*, which was grown in

potato-dextrose broth. Fifty milliliter aliquots of the nutrient solutions were placed in Erlenmeyer flasks and inoculated with approximately equal amounts of the organisms. Two series were run, a short series of 17 fungi, which had been grown at room temperature for 2 weeks, and a long series of 26 fungi, which had grown for 4 weeks. At the end of that time each culture was filtered and the filtrates were poured into 10-ml. vials into which 21-day-old tomato seedlings were placed. Six seedlings were used for each filtrate and all were illuminated by a 100-watt lamp 18 inches above the table. Four of the 5 vascular parasites in the "2-week cultures" produced

TABLE 1.—*Wilt reactions of tomato seedlings in filtrates of various fungi*

Organism	Reaction to filtrate from	
	2-week culture	4-week culture
Fusarium lini	Wilted	Wilted
Fusarium eumartii	Turgid	Turgid
Fusarium conglutinans	Wilted	Wilted
Fusarium elegans	.....	Wilted
Fusarium niveum	Wilted	Turgid
Fusarium lycopersici	.....	Wilted
Fusarium pisi	.....	Turgid
Verticillium albo-atrum	Wilted	Wilted
Fusarium gramineum	.....	Turgid
Papulospora sp.	Wilted	Wilted
Helminthosporium avenae	Wilted	Wilted
Helminthosporium sativum	Turgid	Turgid
Aspergillus clavatus	Turgid	Turgid
Aspergillus ochraceus	Wilted	Turgid
Aspergillus niger	Wilted	Turgid
Perconia byssoides	Wilted	Wilted
Styansus stemonitis	Wilted	Wilted
Penicillium expansum	Wilted	Wilted
Cladosporium herbarum	Turgid	Turgid
Colletotrichum phomoides	Turgid	Wilted
Cephalosporium roseum	Wilted	Wilted
Arachniotus trisporus	.....	Wilted
Arachniotus sp.	.....	Turgid
Rhizoctonia solani (isolate 1)	.....	Wilted
Rhizoctonia solani (isolate 2)	.....	Turgid
Ustilago zeae	.....	Wilted

toxins, while 5 of the 8 in the "4-week cultures" produced them. Eight of the 12 remaining fungi in the "2-week cultures" produced toxins, while 10 of 18 fungi in the "4-week cultures" produced them (Table 1). Thus, it is evident that many fungi can produce toxins that cause wilting in tomato seedlings, when such fungi are grown on artificial media. An examination of table 1 shows that even different isolates of the same species may vary in toxin production; one isolate of *Rhizoctonia solani* produced toxins and the other did not. The toxicity of the filtrate also may vary with the age of the culture, as in the case of *Colletotrichum phomoides*. The 2-week-old culture, for example, was unable to cause wilting, whereas the "4-week cul-

tures'' caused wilt of all 6 seedlings. The results obtained with *Aspergillus* sp., however, were difficult to interpret because 2 of the fungi produced toxins in the ''2-week cultures,'' but did not produce them in the ''4-week cultures.'' This discrepancy might be explained by differences in the test seedlings. Hursh (26) found that the wilting ability of toxins will vary with the age of the plant they act upon, hardiness of the stem, and other factors. It is possible that the seedlings used in filtrates from the ''4-week cultures'' were hardier than those used for the ''2-week cultures,'' although an attempt was made to employ a uniform group of seedlings in all tests. Despite these discrepancies, the experiment indicates that the production of toxins in culture is in itself not sufficient evidence that toxins are produced in plants infected with vascular parasites. The evidence that such deleterious substances cause wilting would be greatly strengthened if their presence could be demonstrated in the wilted plants.

TOXINS IN TRACHEAL FLUIDS

In view of the fact that the results reported in a previous paper (18) had indicated the presence of deleterious substances in the tracheal fluids of wilted plants, it was thought important to repeat these experiments and make further studies on tracheal fluids. Two hundred Bonny Best tomato plants were inoculated by the Wellman technique (55) when about 18 inches high. An equal number of plants were grown as checks under the same conditions, except that they were transplanted without inoculation. The inoculated plants wilted in about 3 weeks, while the noninoculated checks remained turgid. All the plants were then extracted by the technique and apparatus described previously (18). Thirty-five seedlings were used in the assay, 15 for the fluids from wilted plants, 15 for the fluids from the turgid plants, and 5 as absolute checks in distilled water. All the seedlings in the ''wilt fluids'' lost turgidity within 2 hours, whereas those in the ''normal fluids'' and in distilled water remained turgid, even after 12 hours (Table 2). Further studies also were made on plants that had wilted because of insufficient soil moisture, but their extracts exerted no deleterious effects on the test seedlings.

Similar studies also were made on plants that had chronic wilt, in which there is not a sudden loss of turgidity. Instead, the leaves gradually yellow in basipetalous successions and eventually become brittle. Sometimes the plant does not die but remains dwarfed and unthrifty. This condition usually occurs when the environment is unfavorable for the disease, when the host is somewhat resistant, or when the pathogen is not virulent. An assay for deleterious substances was made on the vascular fluids of such plants. The plants and the pathogen were handled in the usual manner, except that the temperatures were somewhat lower, about 65° C. rather than 80° C., and the soil was kept saturated. The checks were grown under similar conditions. The typical chronic wilt appeared in the inoculated plants,

which were then used for the extraction. Sixteen seedlings were used in the assay, 6 for the wilt fluid, 6 for the normal fluid, and 4 for distilled water. Five of the 6 seedlings in the wilt fluid became flaccid after 2.5 hours; the remaining one was only slightly wilted, even after 12 hours. All the plants in the normal fluids and in water remained turgid for at least 12

TABLE 2.—*Time required for the wilting of seedlings in tracheal fluids from tomatoes inoculated with Fusarium bulbigenum var. lycopersici and from noninoculated plants*

Fluid[a]	Condition of seedlings[b] from 1 to 12 hours after placement in fluid											
	1	2	3	4	5	6	7	8	9	10	11	12
WF_1	SW	W	W	W	W	W	W	W	W	W	W	W
WF_2	SW	W	W	W	W	W	W	W	W	W	W	W
WF_3	SW	W	W	W	W	W	W	W	W	W	W	W
WF_4	SW	W	W	W	W	W	W	W	W	W	W	W
WF_5	SW	W	W	W	W	W	W	W	W	W	W	W
WF_6	T	SW	T	W	W	W	W	W	W	W	W	W
WF_7	T	SW	T	W	W	W	W	W	W	W	W	W
WF_8	T	SW	T	W	W	W	W	W	W	W	W	W
WF_9	T	SW	T	W	W	W	W	W	W	W	W	W
WF_{10}	T	SW	T	W	W	W	W	W	W	W	W	W
WF_{11}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{12}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{13}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{14}	T	T	SW	W	W	W	W	W	W	W	W	W
WF_{15}	T	T	SW	W	W	W	W	W	W	W	W	W
NF_1	SW	T	T	T	T	T	T	T	T	T	T	T
NF_2	SW	T	T	T	T	T	T	T	T	T	T	T
NF_3	SW	T	T	T	T	T	T	T	T	T	T	T
NF_4	SW	T	T	T	T	T	T	T	T	T	T	T
NF_5	SW	T	T	T	T	T	T	T	T	T	T	T
NF_6	T	T	SW	T	T	T	T	T	T	T	T	T
NF_7	T	T	T	T	T	T	T	T	T	T	T	T
NF_8	T	T	SW	T	T	T	T	T	T	T	T	T
NF_9	T	T	T	T	T	T	T	T	T	T	T	T
NF_{10}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{11}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{12}	T	T	T	SW	T	T	T	T	T	T	T	T
NF_{13}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{14}	T	T	SW	T	T	T	T	T	T	T	T	T
NF_{15}	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_1	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_2	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_3	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_4	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_5	T	T	T	T	T	T	T	T	T	T	T	T

[a] WF indicates fluids from wilted plants; NF, fluids from normal noninoculated plants; H_2O, water checks.

[b] T, turgid; SW, slightly wilted; W, wilted.

hours (Table 3). From these results we can conclude that a toxin is present in the tracheal fluids of tomatoes infected with *Fusarium bulbigenum* var. *lycopersici* and that it is definitely associated with the presence of the pathogen. Since the toxins are also present in chronically wilted plants, Ahmet's (1) hypothesis that the host may adjust itself somewhat to the presence of the pathogen seems tenable.

Properties of the Toxins

Three properties of the toxins were investigated; the stability in storage, the ease of oxidation, and the thermolability. Vascular fluids that had been stored under nitrogen at 18° C. did not lose their toxicity for tomato seedlings, even after 120 days. The oxydizability of the toxin was determined by bubbling a stream of oxygen through 3 1-ml. samples of "wilt fluids" for 2 hours. "Normal fluids" also were saturated with a stream of oxygen to ascertain whether any toxic compounds were formed during this process. Untreated "wilt fluids" were used as checks and no significant changes

TABLE 3.—*Time required for wilting of seedlings in tracheal fluids of chronically wilted plants*

Fluid[a]	Condition of seedlings[b] from 1 to 12 hours after placement in fluid											
	1	2	3	4	5	6	7	8	9	10	11	12
WF_1	T	W	W	W	W	W	W	W	W	W	W	W
WF_2	T	T	W	W	W	W	W	W	W	W	W	W
WF_3	T	W	W	W	W	W	W	W	W	W	W	W
WF_4	T	SW	SW	SW	SW	SW	SW	SW	SW	SW	SW	SW
WF_5	T	W	W	W	W	W	W	W	W	W	W	W
WF_6	T	T	T	W	W	W	W	W	W	W	W	W
NF_1	T	T	T	T	T	T	T	T	T	T	T	T
NF_2	T	T	T	T	T	T	T	T	T	T	T	T
NF_3	T	T	T	T	T	T	T	T	T	T	T	T
NF_4	T	T	T	T	T	T	T	T	T	T	T	T
NF_5	T	T	T	T	T	T	T	T	T	T	T	T
NF_6	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_1	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_2	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_3	T	T	T	T	T	T	T	T	T	T	T	T
H_2O_4	T	T	T	T	T	T	T	T	T	T	T	T

[a] WF indicates fluids from wilted plants; NF, fluids from noninoculated plants; H_2O, water checks.

[b] T, turgid; SW, slightly wilted; W, wilted.

resulted from oxygenating the wilt fluids. All the seedlings in both the treated and the nontreated wilt fluids wilted within 1.5 hours, whereas those in the oxygenated normal fluids remained turgid even after 12 hours.

The results of the temperature studies were more difficult to interpret. "Wilt fluids" and "nontoxic fluids" were placed in test tubes that were then filled with nitrogen and sealed. Some of the tubes were heated to 100° C., others to 250° C. for 15 minutes; still others were held at room temperature to serve as checks. The tubes, heated to 100° C., contained a fine suspension that did not settle out on standing, while in the tracheal fluids, which had been heated to 250° C., there was much greater denaturation and a copious precipitate. Only the clear supernatant liquid of the latter was used in the assay. Both heated liquids had a sour odor, which was very strong in the tubes that had been heated to 250° C. There were no consistent differences in the time for wilting of seedlings in the fluids that had been heated to 100° C. and the checks; wilting occurred within 1

hour. But one could not make certain that the original toxin was stable and had caused the wilting because the colloidal suspension present might have precipitated in the vessels of the seedlings and plugged them. The supernatant liquid from tracheal fluids that had been heated to 250° C. did not bring about wilting of the test seedlings, whereas the non-heated fluids wilted tomato seedlings within 2 hours.

Because various investigators had indicated that inorganic chemicals may be the toxic components of the tracheal fluids of diseased plants, the tracheal fluids were analyzed spectrographically and the emission element content compared to those of normal plants. Four replicates of each fluid were analyzed but no perceptible differences for any of the emission elements were obtained.

THE PHYSIOLOGY OF WILTING

Many field observations indicate a correlation between the prevalence of wilt and hot dry weather, which tends to increase transpiration. But there are very few reports available in which the transpiration of the diseased plants has been controlled for experimental study. In the present investigations transpiration was controlled in two ways: first, by mechanically preventing evaporation; and second, by the use of a saturated atmosphere. The mechanical prevention of transpiration was accomplished by coating all the exposed surfaces of the test seedlings with vaseline before placing them in the vials containing the wilt fluids. Twenty seedlings were coated in this manner and 20 noncoated ones were used as checks. All the seedlings were placed under electric lamps, at a temperature of 32° C. and a relative humidity of 52 per cent. None of the treated seedlings wilted within 18 hours, whereas the nontreated seedlings lost turgidity within 2 hours. In other experiments seedlings were placed in a saturated atmosphere over a layer of water in a desiccator. Fifteen of the seedlings in the toxic fluids were placed in the moist chamber and 15 were left exposed on the laboratory table; both sets were illuminated by two 100-watt bulbs, which gave an illumination of 405 foot candles. The temperature varied between 26° and 28° C. None of the seedlings in the desiccator wilted, while all those that were exposed in the rather hot, dry air became limp (Table 4). To further test the efficacy of a saturated atmosphere in preventing wilt, 10 seedlings that had wilted under conditions of low humidity were placed in a moist chamber while still in the toxic tracheal fluids and 10 were left in the same environment that had brought on the wilting. All seedlings in the moist chamber recovered within 12 hours, while the others dried without ever regaining turgidity. When the recovered seedlings were removed from the moist chamber to the table, wilting recurred. If again placed in the humid environment, recovery followed; and a subsequent removal again resulted in a loss of turgidity.

Similar experiments were made in the greenhouse with plants that had been inoculated with *Fusarium bulbigenum* var. *lycopersici*. The seedlings

were grown in flats and inoculated when they were about 5 weeks old, after which they were immediately transplanted to 4-inch pots. They were kept in the shade for a week to allow for recovery from the injury of transplanting, then placed on the greenhouse bench. Twenty-four of the pots were placed under bell jars and 25 were left on the bench uncovered. In addition 10 noninoculated seedlings were placed under bell jars as checks. All plants were watered daily, and those under bell jars had large guttation drops hanging from the leaves because of the humid conditions. Of the

TABLE 4.—*Time required for wilting of seedlings in toxic tracheal fluids under different humidities*

Atmosphere	Fluid[a]	Condition of seedlings[b] from 1 to 10 hours after placement of fluids									
		1	2	3	4	5	6	7	8	9	10
Unsaturated atmosphere	WF_1	T	W	W	W	W	W	W	W	W	W
	WF_2	W	W	W	W	W	W	W	W	W	W
	WF_3	W	W	W	W	W	W	W	W	W	W
	WF_4	T	T	W	W	W	W	W	W	W	W
	WF_5	W	W	W	W	W	W	W	W	W	W
	WF_6	W	W	W	W	W	W	W	W	W	W
	WF_7	W	W	W	W	W	W	W	W	W	W
	WF_8	T	W	W	W	W	W	W	W	W	W
	WF_9	T	T	W	W	W	W	W	W	W	W
	WF_{10}	T	T	W	W	W	W	W	W	W	W
	WF_{11}	W	W	W	W	W	W	W	W	W	W
	WF_{12}	T	T	W	W	W	W	W	W	W	W
	WF_{13}	T	W	W	W	W	W	W	W	W	W
	WF_{14}	W	W	W	W	W	W	W	W	W	W
	WF_{15}	W	W	W	W	W	W	W	W	W	W
Saturated atmosphere	WF_{16}	T	T	T	T	T	T	T	T	T	T
	WF_{17}	T	T	T	T	T	T	T	T	T	T
	WF_{18}	T	T	T	T	T	T	T	T	T	T
	WF_{19}	T	T	T	T	T	T	T	T	T	T
	WF_{20}	T	T	T	T	T	T	T	T	T	T
	WF_{21}	T	T	T	T	T	T	T	T	T	T
	WF_{22}	T	T	T	T	T	T	T	T	T	T
	WF_{23}	T	T	T	T	T	T	T	T	T	T
	WF_{24}	T	T	T	T	T	T	T	T	T	T
	WF_{25}	T	T	T	T	T	T	T	T	T	T
	WF_{26}	T	T	T	T	T	T	T	T	T	T
	WF_{27}	T	T	T	T	T	T	T	T	T	T
	WF_{28}	T	T	T	T	T	T	T	T	T	T
	WF_{29}	T	T	T	T	T	T	T	T	T	T
	WF_{30}	T	T	T	T	T	T	T	T	T	T

[a] WF indicates tracheal fluids obtained from diseased wilted plants.
[b] T denotes turgid and W denotes wilted.

25 tomato plants exposed on the greenhouse bench, 7 wilted in ten days, 5 more in 12 days, and the remaining within 14 days. All the inoculated plants under the bell jar remained turgid for 28 days. Thereafter, they gradually wilted; and at the end of an 8-week period only 4 of the original 24 plants were still turgid. But, even the tomato plants that survived had an unthrifty appearance, and when removed from the bell jar to the greenhouse bench they too died. Although none of the noninoculated plants under the bell jars wilted, they also appeared unthrifty and the waterlogged soil and saturated atmosphere seemed detrimental to their vigor.

From these results it is apparent that the toxin disturbs in some way the relation between the water intake of the plants and their loss of water by transpiration. As long as transpiration is reduced the cells do not lose their turgor. The fact that even those inoculated plants that had been kept under bell jars wilted eventually might be explained by the general detrimental effect of the water-logged soil and saturated atmosphere on the vigor of the plants. Noninoculated plants also did not thrive under similar conditions. Thus, the combined effect of the presence of toxin and adverse environment may have brought about the death of inoculated plants.

Toxins and Permeability

As suggested by some earlier investigators, toxins resulting from an infection by *Fusarium bulbigenum* var. *lycopersici* might alter the permeability of the host cells, thereby upsetting the water balance of the host. Experiments were, therefore, set up to measure such changes in cell permeability. Tracheal fluids were obtained from inoculated and noninoculated

TABLE 5.—*Time of deplasmolysis for cells in tissue sections placed in tracheal fluids from infected and noninfected plants*

Fluid[a]	Minutes	Fluid[a]	Minutes
IWF_1	3	NWF_1	7
IWF_2	4	NWF_2	5
IWF_3	4	NWF_3	4
IWF_4	4	NWF_4	5
IWF_5	4	NWF_5	17
IWF_6	5	NWF_6	17
IWF_7	4	NWF_7	14
IWF_8	4	NWF_8	17

[a] IWF denotes tracheal fluids of infected plants. NWF denotes fluids of plants wilted because of insufficient soil moisture.

plants in the usual manner, and fluids of normal noninoculated plants that had wilted because of lack of soil moisture were used as absolute checks. The latter control was important; otherwise, any changes in the permeability of the infected, wilted plants, when compared to the permeability of normal turgid plants might be due to the process of wilting itself and have no relation to the presence of the parasite in the vascular tissue.

All measurements of changes in permeability were made on the pith cells from petioles of actively growing young plants. Using calcium chloride solutions of various concentrations, the osmotic pressure of normal noninoculated plants was determined as 10.6 atmospheres, which was equivalent to a 0.403 molar solution isotonic with the pith cells. A hypertonic 0.806 was used for plasmolysis and a hypotonic 0.303 molar solution for deplasmolysis, and the time necessary for deplasmolysis was used as a criterion of cell permeability. Since absolute permeabilities would give no information pertinent to the problem, these values were not calculated. Tissue sections were placed in the tracheal fluids from both infected and noninfected plants for 2 hours and then removed to the hypertonic solution

for 20 minutes. After removing the excess of reagent, the sections were placed in the isotonic solution and observed for time of deplasmolysis. At least 100 cells were observed in each section. All but one of the sections, IWF_6, that had been placed in the toxic fluids, required less time for deplasmolysis than those in the normal fluids (Table 5). The results of the foregoing experiments indicate that the toxin produced in infected tomato plants increases the permeabilities of the cells. Such an increase in permeability could readily play an important role in causing tomato plants infected with the *Fusarium* to wilt.

PERMANENCE OF THE TOXIC ACTION

Although wilting was reversible when conditions that hindered transpiration prevailed, these results do not indicate whether or not a permanent change in permeability had taken place. For this reason a series of experiments was made in which plants were removed from the toxic fluids at various periods after wilting had begun, and placed in distilled water. The periods of time after wilting set in varied from 15 min. to 5 hr. All the plants recovered except those that had already started to dry out, which indicated a permanent disorganization of the tissues. Under the conditions of the experiment the maximum time that seedlings could remain in the wilted state and still recover turgidity was 4.25 hours. Hursh (25) reported that after wilted cabbage seedlings had recovered in water they did not again wilt when returned to the toxic filtrates, and Chester (7) interpreted this phenomenon as an example of acquired resistance in plants. Similar experiments were performed with tomato seedlings, both in fungus-culture filtrates and in toxic tracheal fluids. Twenty seedlings that had wilted in the tracheal fluids and recovered in the water within 4 hours again lost their turgidity when returned to those toxic fluids. Thirty-nine seedlings responded in a corresponding manner to toxic filtrates from fungus cultures. In no case was there any evidence of acquired resistance in the tomato seedlings.

Attempts were made to discover whether plants infected by the pathogen will recover when the supply of toxin is interrupted. Plants that had been inoculated and grown in the greenhouse until wilting began were cut a few inches below the uppermost petiole and the tops placed in water. At this period the upper portion of the stem contained no mycelium, and thus the top could obtain no more toxin during the time it was in the water. All these plants recovered. But those plants whose stems were cut at the soil line and then placed in water never recovered. The fungus was well established in the lower portion of the stem and could therefore continue to produce more toxin, which resulted in the death of the plant. The recovery of the tomato plants when removed from the influence of the fungus or from the toxic filtrate indicates that the toxin itself probably does not permanently disorganize the cell. The plant dies only after the disorganization of the water relations has proceeded far enough to bring about other changes

in the physiology of the host. If the effect of the toxin is removed before that time, the tissues regain their normal function. An uninterrupted toxin supply seems necessary before permanent disorganization of the protoplast occurs.

DISCUSSION

Although toxins long have been suspected of playing an important role in vascular wilts, there has been no proof hitherto of their presence in the tissues of infected plants. The results of the present investigation and of a previous one by the writer, demonstrated such deleterious substances in the tracheal fluids of tomato plants wilted by *Fusarium bulbigenum* var. *lycopersici*. Moreover, the active constituent is definitely associated with the presence of the pathogen in the vascular tissues, for no toxins could be obtained in noninoculated plants that wilted because of lack of soil moisture. It was not possible, however, to determine whether the toxin is a metabolic product of the fungus or a reaction product of the host due to the presence of the fungus. In addition to causing wilting of inoculated plants the toxin apparently brings about a discoloration of the xylem similar to that observed by Rumbold (45) when deleterious substances were injected into trees. Such symptoms also were observed by Overton (39) and Haskell (20) when stems were injured by steam, in which case Overton postulated that the toxins were a product of the dead cells. In the present investigation the toxins seem to be carried from the infected portion of the xylem to the leaves by the transpiration stream, as has been demonstrated by Free (17) for toxic minerals from the soil, because of the evaporation of the transpired water from the leaves. Free believed that the concentration of the toxin is increased until a derangement of the cell takes place.

The toxins in tomato appear to disturb the water balance of the host plant. Normally, there is a critical balance between the intake and the loss of water by the plant. A similar ratio must also exist in the leaf tissues, so that wilting can occur either because of increased transpiration or decrease in absorption of water. The toxins produced in the plant would tend to reduce that ratio below the critical level. Apparent wilting may conceivably be due also to a loss of turgidity as a result of the excretion of water from living cells into the intercellular spaces. However, wilting never occurred in tomato plants when they were in the toxic fluids, provided transpiration was prevented; and plants that had previously wilted regained their turgidity under such conditions. These results help explain the prevalence of vascular wilts during hot, dry periods when the transpiration is so high and the absorption so low that the upset in water relations of the plant is easily carried beyond the point necessary for turgidity. They also may explain the diurnal wilting and recovery reported in diseased plants. These hosts might wilt in the daytime as a result of the accelerating effect of the toxin on the movement of water out of the leaves; they recover their turgidity in the night when the transpiration is decreased so that the absorption transpiration ratio is raised above the critical level. This peri-

odic wilting lasts only a few days because in infected plants the parasite constantly produces toxin, which eventually could reduce the ratio so much that even the nightly decrease in transpiration would not suffice to raise it above the critical level. Somewhat analogous conditions were obtained experimentally when wilted tomato seedlings were allowed to remain in toxic filtrates for a period of 5 hours; when transferred to the moist chamber the seedlings did not regain turgidity, whereas those plants that were transferred sooner recovered temporarily.

The results obtained by measuring cell permeabilities indicate that the toxins present in the tracheal fluids of diseased tomato plants increase the permeability of the cell to water. These results are in agreement with the observations of Thatcher (50) on tomato plants that had wilted in "fungal filtrates." He observed a small increase in permeability for cells that had wilted when compared to the cellular permeability of turgid plants. There is a possibility that the increased permeability he observed may have been the result of the process of wilting and not its cause, for similar processes, such as drought hardening, also increase cell permeability (29). In the current investigation, to overcome this difficulty, small sections of healthy tissue were immersed immediately in plant fluids where wilting was impossible; and comparisons of cell permeabilities were between those placed in tracheal fluids from diseased plants and those placed in fluids from plants wilted because of insufficient soil moisture. Because of the increased transpiration at the time of wilting of pea plants, infected with *Fusarium pisi*, Linford (31) also postulated an increased permeability in the fusarial wilt of peas. Yet, his data indicated that, prior to actual wilting, there is a reduction in transpiration. Such a reduction might not necessarily indicate that there is also an initial decrease in permeability, for it is known that incipient wilting is accompanied by a loss of turgor that would cause the guard cells to close (38) and thereby reduce transpiration. When true wilting occurs the guard cells open wide and transpiration sharply increases (36).

The recovery of wilted seedlings when placed in water can be accounted for by the assumption of an inactivation of the toxin by the tissues. If the activity of the toxin permanently changed the permeability of the cell membranes the transference of wilted plants to water should not result in their recovery. Since the plants do regain their turgidity, the effect of the toxins is not lasting; and one might assume that the toxins that caused the initial wilting may have been inactivated or combined in some manner in the host cells. A continuous supply of toxin is, therefore, necessary to keep the plant in a wilted condition. This condition results when seedlings are kept in the toxic tracheal fluids and when the pathogen in the infected plants continuously produces the toxins that are transported to the leaves.

Very little is known concerning the nature of the tracheal toxins other than their stability to oxygen and perhaps to high temperatures. Spectrographic analyses have shown no correlation between the emission element

content of the tracheal fluids and the toxicity of these fluids to tomato seedlings. Greater promise for determining the toxic fraction lies in the field of bio-organic chemistry. Special attention should be given to the nitrogen, and amine content of the toxic and nontoxic tracheal fluids to establish whether the toxic amine fraction found by Ahmet (1) and Grossman (19) in the "fungal filtrates" is also present in the tracheal fluids of diseased tomato plants.

SUMMARY

A dense mycelial growth of *Fusarium bulbigenum* var. *lycopersici* was found in approximately 50 per cent of the vessels in the root and lower stem of wilted tomato plants, but no isolations of the fungus could be made from the top of the stem.

Attempts to measure the effect of this occlusion on the movement of water through tomato stems were unsuccessful.

Toxins are produced in culture by a large variety of fungi, including vascular parasites, root-rotting and leaf-spotting organisms, as well as saprophytes.

The wilting of tomato plants infected with *Fusarium bulbigenum* var. *lycopersici* is correlated with the presence of a toxin in the tracheal fluid of the host. Infections that result in chronic wilt also bring about the production of toxins in the tracheal fluids.

The toxins obtained from diseased tomato plants are stable to oxidation, and spectrographic analysis revealed no significant differences between the emission-element content of fluids from the diseased and from healthy plants.

The toxin increases the permeability of cells of the host plant.

Plants that have been wilted by the toxic fluids will recover when placed in distilled water, while their subsequent removal to toxic fluids will again cause the loss of turgidity.

The toxin disturbs the normal water relations of the plant. When the transpiration of seedlings that had been placed in the toxic tracheal fluids is prevented, wilting does not occur. Even wilted seedlings eventually regain their turgidity under these conditions.

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ACUTE AND CHRONIC SYMPTOMS IN THE TOBACCO RING-SPOT DISEASE

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INTRODUCTION

Reports by Valleau (8), by McKinney (1) and by McKinney and Clayton (4) have emphasized that the tobacco ring-spot disease is characterized by two phases that may be regarded as being essentially acute and chronic, respectively. It has been reported also that the acute phase may be brief, followed by an extended chronic phase (1), or the acute phase may continue from infection to maturity of the plant (1); that these disease reactions are influenced by the age of the plant, the amount of inoculum introduced, and growth conditions (1, 4); that tobacco is a host of medium susceptibility (1); that Early White Spine cucumber is more susceptible than tobacco (4); that the chronic phase is attended by mosaic symptoms more regularly in Early White Spine cucumber than in tobacco (4); and that the causal agent may be regarded as a mosaic virus (4).

The fact that the chronic phase of the ring-spot disease in tobacco usually is not attended by striking visible symptoms has led to the view that the progress of the disease differs fundamentally from the progress of the mosaic diseases. However, this view is not supported by all of the facts. One of the writers, McKinney (1), has taken the view that natural resistance in the commercial tobaccos is the chief basis for the usual lack of striking symptoms in the leaves that appear during the chronic phase, and for the delay in the onset of the chronic phase when the older plants are inoculated.

These studies were carried out to provide information on several factors influencing disease expression, *i.e.*, age of plants, culture conditions, methods of inoculation, host genotype and host species. Limited tests were carried out to determine the effects of the virus on the weight of leaves or leaves and stems.

MATERIALS AND METHODS

The studies reported here were conducted chiefly with the following varieties and collections of tobacco; Wisconsin-Havana Seed, Samsun (Turkish), T.I. 448A from Colombia and T.I. 706 from Honduras. Most of the work with ring spot was done with T.I. 448A and other selections from this number for the reason that they are susceptible to it and are resistant to tobacco mosaic. This made it possible to avoid the troublesome confusion with the latter disease in the field.

Tobacco ring-spot virus supplied by W. C. Price was used throughout the studies. Inoculations were made by various methods indicated throughout the text. The most active virus was obtained from the leaves of infected cucumber, *Cucumis sativus* L. var. Early White Spine.

[1] Acknowledgment is due Matthew Koerner for assistance in conducting the tests.

Plants were cultured in the greenhouse, in pots, large pails, and large wooden boxes. In the field, the tests were conducted in the usual manner current in experimental work with tobacco. The soil was well suited to tobacco culture and a balanced fertilizer was applied at the beginning of the season.

Virus assays were conducted on plants of bean, *Phaseolus vulgaris* L., var. Scotia and cucumber, var. Early White Spine. Extracts were applied to primary leaves or on cotyledons by means of the carborundum-wipe method. Temperatures were maintained near 33° C. for 24 to 48 hours after inoculation. After this period the ordinary greenhouse temperatures were maintained.

Primary lesions tend to be irregular on both test plants and, therefore, most comparisons were based on the number of plants developing secondary, acute symptoms. Cucumber was favored as the test plant as it provided large quantities of active virus from the chronic mosaic leaves, whereas in the bean the systemic infection induces necrosis and death. These tissues and the primary lesion tissue yield less virus than the leaves from cucumber plants.

RESULTS ON TOBACCO

Influence of Age of Plants and Culture Methods on Symptom Expression

Early in the studies on the ring-spot disease, it was observed that irregularities occurred in symptom expressions from test to test. Many of these observations suggested that the age of the plants and culture conditions might explain some of these irregularities, especially as it appeared that tobacco does not rank very high as a suscept.

An experiment was carried out to determine the effects of age and the vigor of growth of the plant on the expression of primary acute (local necrotic lesions and ring spots) and secondary acute symptoms (systemic necrotic lesions, ring spots, and oak-leaf patterns). Three types of tobacco were used. Two ages and three ages were grown in three different sizes of soil containers as indicated in table 1.

Fertile composted soil was used throughout. The plants were grown in a greenhouse shaded with whitewash during the late summer. All plants were inoculated at the same time (Aug. 15) with nondiluted virus extract from heavily infected leaves of Scotia bean plants. Each tobacco plant was inoculated by wiping 4 top leaves, the topmost one ranging from 65 to 90 mm. long. There were 4 plants in each test group. At the time of inoculation the plants growing in the 6-inch clay pots were definitely pot-bound, those in the 8-inch clay pots were showing slight effects from potbinding, whereas plants growing in the boxes were almost as vigorous as those cultured in the field. The final data were recorded Sept. 6 when some of the plants had started to flower. The results are given in table 1.

TABLE 1.—*Data showing the influence of age and plant vigor on the expression of primary and secondary acute symptoms caused by the ring-spot virus in tobacco*

Collection or variety of plant used	Age of plants in days	Type of soil container used, the number of primary lesions per 100 cm.² of leaf surface, the percentage of plants with secondary acute symptoms and the degree of expression								
		6-inch pots			8-inch pots			Boxes[a]		
		Primary lesions	Secondary acute symptoms		Primary lesions	Secondary acute symptoms		Primary lesions	Secondary acute symptoms	
			Per cent of plants	Degree of expression		Per cent of plants	Degree of expression		Per cent of plants	Degree of expression
		Number			*Number*			*Number*		
T.I. 706	78	0	0	.....	0	0	.....	5.9	50	Mild
"	92	0	0	.....	0	0	.....	2.5	25	Trace
Samsun (Turkish)	78	0	0	.....	0.65	25	Mild	12.2	100	Very severe
	92	0	0	.....	1.30	25	Trace	5.5	100	Severe
	102	0	0	.....	0.57	0	.....	7.3	100	Severe
T.I. 448A	75	0	0	.....	4.56	25	Mild	29.3	100	Severe
	88	0	0	.....	0.81	25	Mild	11.7	100	Severe
	102	0	0	.....	0.73	25	Trace	12.53	50	Trace

[a] These boxes contained 1¼ cu. feet of soil per plant.

The data show that the pot-bound (retarded) plants were most resistant to the expression of acute symptoms. In all cases the most vigorous plants (cultured in boxes) showed the most severe symptoms. In no instance were the oldest plants the most susceptible. These results have been verified many times in numerous tests bearing on other phases of the problem.

Nonthrifty and older plants that manifested no signs of primary necrotic lesions sometimes did manifest obscure signs of local infection on the wiped leaves. Examination of such leaves by diffuse transmitted light, especially in a darkened room, revealed occasional light-green circular zones as illustrated in figure 1.

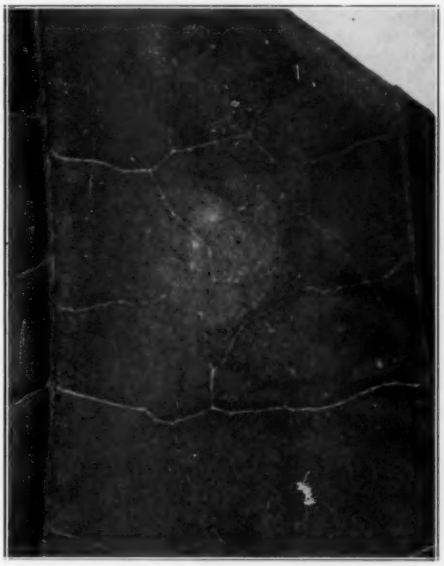

FIG. 1. Portion of leaf of Samsun tobacco illustrating one of the occasional primary light-green zones occurring when leaves on pot-bound plants are wiped with tobacco ring-spot virus. Photographed by transmitted and reflected light. ×2.

In table 1 it will be observed that T.I. 706 was more resistant than T.I. 448A and Samsun. Many tests have shown this resistance in T.I. 706. The difference in resistance between T.I. 448A and Samsun has been less consistent, but from observations made over a period of time, it appears that T.I. 448A is the more susceptible. This is an especially desirable tobacco for the ring-spot studies because it is highly resistant to the common-mosaic virus and its mutants (2, 3). The chlorotic patching, which Valleau (8) reported, has not been observed in the chronic diseased leaves of Samsun tobacco plants grown in these tests.

Samsun and T.I. 706 are highly susceptible to the common-mosaic virus and its mutants, in that they sustain a very high level of virus synthesis. Both tobaccos are highly tolerant, however, especially in the chronic phase and they complete their life cycles under ordinary culture conditions, in

spite of reductions in the chlorophyll. The secondary acute symptoms induced by the ring-spot virus in tobacco are frequently similar to those induced by alfalfa-mosaic virus 1B (10).

When young tobacco plants are inoculated with sufficiently active virus, the acute phase of the ring-spot disease tends to involve but 1 to 4 consecutive leaves above the point of inoculation and the onset of the chronic phase is hastened and, therefore, this phase involves the great majority of the leaves of the mature plant. On the other hand, when older vigorous plants are inoculated, the number of leaves on the mature plant that show the acute and chronic phases may be about equally divided or the acute phase may continue on all the leaves until the plant matures without the chronic phase

TABLE 2.—*The influence of age of tobacco plants inoculated with ring-spot virus, on the number of leaves in the secondary acute phase and in the chronic phase of the ring-spot disease in T.I. 448A tobacco. Inoculation was by the wiping method and plants were cultured in adjacent plats in the field... The same lot of inoculum was used throughout*

Plant No.	Leaves with indicated symptoms and "skip" leaves[2] in plants inoculated when					
	42 days old			63 days old		
	Secondary acute symptoms	"Skips"	Chronic symptoms	Secondary acute symptoms	"Skips"[a]	Chronic symptoms
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	1	0	40	35	6	0
2	4	0	38	36	4	0
3	3	0	41	13	6	20
4	2	0	42	19	1	19
5	5	0	36	16	1	22
6	1	0	43	37	0	0
7	3	0	44	24	4	11
8	4	0	38	19	1	11
9	4	0	39	11	0	30
10	3	0	40	33	7	0

[a] See table 4. In this test most of the "skips" were near the zone of inoculation. Leaves below the inoculated leaves were disregarded, as they did not show symptoms.

becoming evident. In either case, however, when the older plants are inoculated, there may be a number of "skips"[2] among the leaves that show the acute symptoms. Representative data in table 2 illustrate this.

The differences in reaction of young and old plants to the virus seems to be accounted for on the basis of resistance that increases with age, as it appears that virus invades the very young tissues and possibly the growing point most readily in young plants.

Effect of Inoculation Methods on the Type of Symptom Expression

Many inoculation tests made by the wiping method have shown that fine carborundum powder (600 grain) materially increases the expression of the primary and the secondary acute symptoms. A typical experiment is cited.

[2] "Skips" are leaves free of acute symptoms but flanked by leaves with acute symptoms.

On August 21, 1941, 55 vigorous healthy plants of T.I. 706 and 6 plants of Samsun, all from 8 to 10 inches tall, growing in a field plat, were inoculated. The virus extract was obtained from freshly collected leaves of T.I. 448A tobacco, all of which had severe acute symptoms of the ring-spot disease. They were clipped and pressed under hydraulic pressure and the extract was used without dilution. Alternate plants in each row of 8 to 10 plants were inoculated without carborundum by wiping the virus extract

TABLE 3.—*Increased infection with the ring-spot virus when carborundum powder was dusted on leaves before wiping with virus extract. Samsun (Turkish) tobacco plants used. The degree of the acute reaction is given for each leaf above the inoculated leaves on each plant to illustrate the irregularities leaf by leaf, especially when the amount of initial virus entering the plant is small. Data were taken at maturity of plants*

Leaves numbered upward from above those inoculated	Degree of secondary acute reaction in each leaf[a] inoculated					
	Without carborundum Plant No.			With carborundum Plant No.		
	1	3	5	2	4	6
25	.....	.....	.....	.....	.....	75
24	.....	0	.....	.....	.....	100
23	.....	0	.....	.....	0	90
22	.....	0	.....	100	25	100
21	.....	0	.....	100	100	100
20	0	0	0	100	75	90
19	0	0	0	100	100	100
18	0	0	0	100	100	100
17	0	0	0	100	90	85
16	80	0	80	100	100	100
15	0	0	0	100	40	30
14	1	0	1	100	100	5
13	5	0	5	100	90	100
12	0	5	0	100	95	25
11	10	0	10	100	100	40
10	0	0	0	100	95	30
9	0	0	0	75	95	1
8	40	0	40	75	60	60
7	0	0	0	40	90	1
6	0	0	0	30	50	1
5	0	0	0	50	5	10
4	0	0	0	20	75	3
3	0	0	0	30	5	1
2	0	0	0	5	0	0
1	0	0	0	25	0	0

[a] The plants inoculated without carborundum and with carborundum developed 7 and 46 primary lesions per plant, respectively. The values in the columns are percentages of severity based on a rating of 100 for a leaf with the most severe reaction as illustrated in figure 2, B.

on 4 top leaves of each plant, which leaves ranged from 4 to 12 inches long. The wiped leaves were rinsed with water. The remaining plants in the row were then inoculated by the carborundum-wipe method and rinsed with water. Thus, except for the carborundum, the inoculation procedure and the inoculum were the same in both sets of plants. Leaves of the same size, within narrow limits, and in the same position, were inoculated throughout.

The advantage gained from carborundum powder is clear from the summarized data in table 3. Although fresh nondiluted virus extract was

used, the number of primary lesions was exceedingly small. This may be explained in part by cool weather conditions that prevailed for a few days after inoculation. Data from the T.I. 706 tobacco plants are not presented, but they were consistent with those obtained from the Samsun plants.

FIG. 2. Leaves of T.I. 448A tobacco showing weak (A) and severe (B) secondary acute symptoms of the ring-spot disease resulting from inoculations with extracts of low and high virus activity, respectively. These leaves were tenth above the inoculated leaves. $\times \frac{1}{2}$.

In table 3 the data for each leaf on 6 Samsun tobacco plants are presented to show the irregularity that occurs in the expression of secondary acute symptoms. These data illustrate the "skip" leaves referred to in table 2. Weak expression of acute symptoms and the number of symptom-free leaves has been associated with inoculation methods that favor the entry of small quantities of virus, and also with old plants and reduced vigor. Weak and severe secondary acute symptoms are illustrated in figure 2.

Another inoculation test was undertaken with a view to getting the greatest possible quantity of the ring-spot virus into the stem tip. In the resistance studies with T.I. 448A against the common-mosaic virus (3) and its yellow-mosaic mutants, it was found that "skip" leaves occurred. Frequently it was found that virus-free leaves were flanked by leaves carrying virus. Experience gained from the study of mosaic-resistant tobacco and related species leads to the belief that "skip" leaves can be regarded as good evidence of certain types of resistance in host plants when optimum culture conditions prevail.

All of the circumstantial evidence indicates that tobacco does not support a very high level of synthesis for the ring-spot virus, and, as a consequence, small amounts of the virus introduced into the plant do not incite a huge production of virus that quickly invades the plant. In order to counteract the natural resistance of the plant—within limits—it is necessary to introduce large amounts of virus in the inoculum.

Observations on the behavior of the ring-spot disease and preliminary assays for virus activity have supported Valleau's (8) view that the expression of the chronic phase probably depends on infection in or very near the primordial meristem. Accordingly, tests were conducted by methods that seemed most likely to force adequate amounts of virus into this region, and thereby favor the early onset of the chronic phase. Since plants of advanced age resist the chronic phase to a greater degree than young plants (table 2), tests were conducted with vigorous healthy plants 56 days old from the time of seeding, to determine if the appearance of the chronic phase can be hastened when the virus is introduced directly into the tip of the stem by means of a fine dissecting needle. Fifteen plants of T.I. 448A were inoculated in this manner with fresh active virus extract from cucumber and a like number of plants were inoculated by wiping the same virus extract on one leaf, from 75 mm. to 105 mm. long, at the top of each plant. Culture was in 6-inch earthen pots in a greenhouse.

In the series inoculated in the stem tips, 3 plants failed to show symptoms, 12 developed acute symptoms on 8 to 11 leaves per plant, and in 10 of these the acute symptoms were very severe. At the end of a 40-day period all 12 infected plants had passed well into the chronic phase. On the basis of leaf counts made in the dissected tips of plants in a similar stage of development as those inoculated, it appears likely that all, or nearly all of the leaves with acute symptoms were formed at the time of inoculation in the series inoculated in the stem tip.

In the series inoculated by wiping, all plants developed acute symptoms; in 13 plants the symptoms became severe, and in 2 plants they were weak. At the end of the 40-day period, the chronic phase had not appeared in any of these plants. The tip inoculation method was used in several tests and found to be erratic. Acute symptoms either failed to appear or they were very mild. Subsequent tests have indicated that the virus should be introduced very close to the growing point.

Since large quantities of virus influence the expression of acute symptoms so greatly (table 3), it was decided to amputate some of the apical leaves in aging plants and then inoculate leaves directly below the amputated zone with very active virus extracts. It seemed reasonable to believe that when the virus was deprived of access to several leaves, part of the large quantity of virus present in the wiped leaves would get into the apical zone sooner than if the larger apical leaves were left intact.

Leaves 3 to 4 mm. long and smaller were allowed to remain on the stem tip and the 5 or 6 leaves below these, which were from 10 to 60 mm. long, were carefully removed. This procedure was carried out on alternate plants in the rows in the field, the remaining plants serving as controls. In all, 70

TABLE 4.—*Data showing the influence of the amount of virus entering the wiped leaves, and also the influence of amputation of 5 or 6 small leaves near stem tips of inoculated plants on the expression of the acute and the chronic symptoms of the ring-spot disease in tobacco. No. T.I. 448A cultured in the field*

Items	Virus from cucumber leaves		Virus from tobacco leaves	
	No leaves amputated	5 or 6 leaves amputated	No leaves amputated	5 or 6 leaves amputated
Plants inoculated (number)	19	18	15	15
Primary lesions per cm.^{2} of leaf surface inoculated (number)	1	1	00.02	00.02
Plants with secondary acute symptoms (number)	19	18	5.0	14.0
Plants manifesting the chronic phase (number)	6	17	0	0
Leaves per plant above amputated leaves (average number)[a]	24.21	29.16	.....	.....
Leaves occurring in the acute phase[b] (per cent)	90.66	55.41	.....	.....
"Skip" leaves appearing in acute phase[c] (per cent)	6.70	00.00	.....	.....
Leaves appearing in the chronic phase (per cent)	9.33	44.58	.....	.....

[a] In the nonamputation control, the leaves corresponding to those amputated are not included in the counts. Leaves on main floral axis are included.

[b] The "skip" leaves were included.

[c] This percentage is based on the number of leaves in the acute phase, but the other percentages are based on the number of leaves above the zone of leaf amputation.

vigorous plants of T.I. 448A, 63 days old from seeding, were used. Inoculation was by the carborundum-wiping method. Four leaves, from 8.0 to 25.0 cm. long, and directly below the amputated leaves, and equivalent leaves on the controls, were inoculated and then rinsed with water. Fresh concentrated virus extract from infected cucumber leaves was diluted in 3 volumes of M/100 phosphate buffer at pH 7.0 (6) and used to inoculate 40 of the plants, and fresh concentrated virus extract from Samsun tobacco leaves in the chronic phase and diluted in 1 part of buffered solution, was used to inoculate the remaining 30 plants.

The results of this test are presented in table 4. The activity of the virus from cucumber was much greater than that of the virus from tobacco.

This is reflected in the number of local infection cites (primary lesions) and in the expression of the secondary acute symptoms.

Where the more active inoculum was used, primary lesions appeared 2 days after inoculation and in the nonamputated controls, the secondary acute symptoms appeared 6 days after inoculation in the leaves that corresponded to those removed in the amputation series. In the amputation series, the secondary acute symptoms appeared in the new apical leaves 10 days after inoculations, and within 2 days the symptoms on these leaves were the most severe ever observed by the writers. For a period of 10 days the secondary acute symptoms were clearly more severe in the amputation

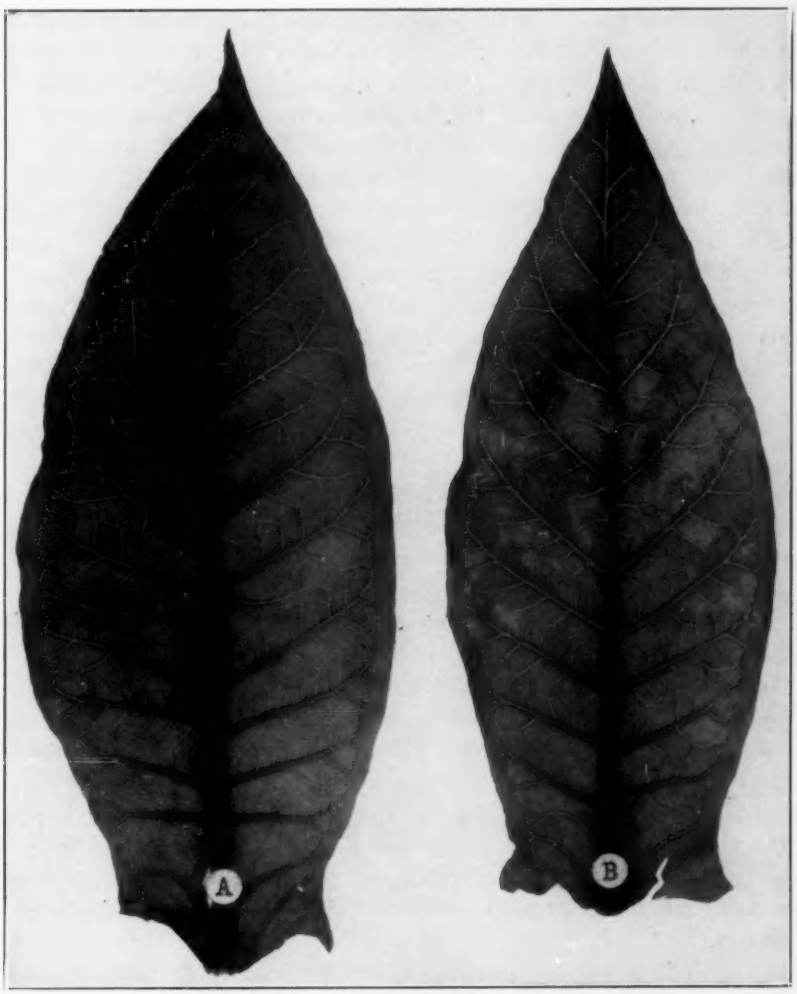

FIG. 3. Leaves of T.I. 448A tobacco, A, healthy and B, with mosaic mottling in the chronic stage of the ring-spot disease developed during cool periods. Photographed with transmitted and reflected light. × ½.

series than in the control. As the plants developed, the severity of the acute symptoms in the amputation series tended to become less in the new leaves, but there were few new leaves with reduced symptoms, as the chronic phase started to appear 10 days later (20 days after inoculation). During periods of cool weather, chlorotic patches and mosaic mottling appeared in leaves 10 to 20 cm. long on the plants that manifested the chronic phase. The chlorotic patches resembled those illustrated by Valleau (8), and the type of patching or heavy mottling that is characteristic of tobacco mild dark-green mosaic. A rather typical mosaic mottling is illustrated in figure 3, B. A distinct mosaic virus could not be isolated from these and similar leaves. Throughout the work it was observed that in plants of T.I. 448A (tobacco) grown in the field, the chlorotic patching and mosaic mottling tended to be most prevalent in the first 4 to 8 leaves with chronic symptoms. In table 4 it will be noted that no "skip" leaves appeared in the amputated series.

Where the less active virus from tobacco was used, the secondary acute symptoms were delayed, appearing 20 days after inoculation. The symptoms were very weak in the lower leaves, but they tended to increase progressively in the new leaves until they were moderately severe in the upper portion of the plants. Chronic symptoms failed to appear in any of the plants, but the data in table 4 show that amputation of the small leaves favored secondary acute symptoms.

In the amputation series inoculated with the highly active virus from cucumber, it is reasonably certain, from gross dissections made on similar plants, that more than half of the leaves that developed acute secondary symptoms were differentiated by the time virus entered the apical zone. Microscopic studies might have revealed a greater number of differentiated leaves.

This test indicates that the proximity of large quantities of the virus to the stem apex favors the expression of the chronic phase of the ring-spot disease. The small leaves directly above the amputated leaves developed the most severe acute symptoms ever noted by the writers, thus a large reservoir of virus was established close to the growing point, making it possible for the virus to surmount some form of natural resistance and enter very young leaves in quantity. The results of this test and the results obtained from the needle inoculations made directly into the stem tips, are in general agreement.

Although it is not the purpose of the present study to determine the relative quantities of ring-spot virus in different tissues, certain preliminary tests have been made, and the results are summarized.

"Skip" leaves varied with respect to the presence of detectable virus. Some contained detectable virus, whereas others did not. In leaves manifesting mild and moderate secondary acute symptoms, most of the normal green tissue is at first free of detectable virus, but later on virus enters some of these areas. In a general way, this resistance in leaves with the secondary acute symptoms of the ring-spot disease is suggestive of the resis-

tance shown in leaves of tobacco T.I. 448A against common-mosaic virus (wild type) and its yellow-mosaic mutants (3). However, the level of resistance is higher against the mosaic viruses than it is against the ring-spot virus.

Tobacco plants with the mild acute symptoms of ring spot apparently have no detectable virus in the stem apex; in plants with the severe acute symptoms, virus was detected in the stem apex, but at much lower concentration than in the ring-spot tissue of the leaves. In plants in the chronic stage, virus-activity tests were made on the stem tips[3] and on a series of leaves dissected from the tip. The virus activity tended to be slightly greater in the stem tips than in the leaves. The onset of the chronic phase may be determined by a relatively high level of virus activity in the stem tip rather than by mere presence of virus in that region of the stem.

Leaves 24 to 40 cm. long and produced during the chronic stage were sampled by means of cork borers. Each small disc was assayed separately on 2 to 10 cucumber seedlings. Thirty-one discs 11 to 19 mm. in diameter from three leaves were tested and virus was present in each. Sixty-six discs 4.5 mm. in diameter were taken from three other nearly mature leaves and tested. All but 5 of these contained virus. These results indicate that invasion is almost solid in the leaves produced during the chronic phase, yet the resistance of this tissue is so high that visible signs of disease are absent at temperatures that favor symptoms in a more susceptible host, such as cucumber, and in tobacco when infected with common mosaic virus.

Owing to a very large experimental error in the methods used for assaying the ring-spot virus, further study is necessary before critical interpretations are possible from assay data. Studies on the apical stem tissues are very difficult in Samsun and T.I. 448A tobacco. Preliminary work indicates that Maryland Mammoth tobacco offers possibilities for more accurate studies because of the unusually large size of the apical zone, and on account of its indeterminate vegetative growth when cultured in a long photoperiod.

Weight of Tobacco Leaves and Plants as Influenced by the Ring-spot Disease

During the season of 1941, 36 plants of T.I. 448A tobacco were grown in the field in three rows of 12 plants each. Four plants in each row were used as noninoculated controls and the other 24 plants were inoculated. The soil was uniform, and well adapted for commercial tobacco culture, and the plat was practically level.

On July 3 when the plants were 25.0 to 35.0 cm. tall, they were inoculated by wiping virus extract on 4 top leaves, 8.0 to 25.0 cm. long. No carborundum was used. The inoculum consisted of an extract from infected primary leaves of Scotia beans diluted in 4 parts of phosphate buffered solution.

The controls remained free of ring spot and other diseases. The inoculated leaves developed primary lesions at the rate of about 1 lesion per

[3] Stem tips included all differentiated leaves up to 20 mm. long.

square centimeter of surface. All plants developed severe secondary acute symptoms, 5 plants failed to show the chronic phase, whereas in the remaining 19 plants, the chronic phase appeared in from 11 to 31 top leaves.

Owing to the severity of the acute reaction, it was not possible to obtain the information desired on the chronic phase. However, the yield data do show the marked reduction caused by the acute symptoms.

Leaves were harvested at three periods during the season, beginning above those inoculated on each plant, when the leaves had attained their full development. Thirty market-type leaves were obtained from each plant in the control and in the inoculated series.

At each harvest the yield (green weight) from the controls was almost double that from the diseased plants. The average total yield in the controls was 1.004 kilograms per plant, and in the infected series it was 0.502 kilograms per plant.

During the season of 1942, 30 plants of T.I. 448A tobacco, grown in the field, were prepared for a yield test on the chronic phase of the disease, but the inoculum used proved to be too weak. All the plants developed primary lesions, but only 24 developed weak secondary acute symptoms, one of which developed the chronic stage late. Six plants gave no signs of systemic infection. Rather than lose the entire season, another test with 30 plants of the same variety of tobacco, similarly grown, was prepared as soon as it became evident that a weak inoculum had been used. Fifteen of these plants in the second series were inoculated. As they were very young when inoculated, they developed the chronic phase quickly. The acute phase involved only 1 to 4 of the lower leaves per plant.

During the season it was noted that the infected plants were not so robust as the controls. As the season advanced, there was some irregularity in the vigor of all the plants, but the controls averaged the more robust. Just before flowering, the plants were harvested and each plant, stem and all leaves, was weighed.

The healthy controls gave an average green weight per plant of 1.483 kilograms, whereas the infected plants gave an average weight per plant of 1.034 kilograms. Although the infected plants manifested no acute symptoms after they were transplanted to the field, they showed a 30.28 per cent reduction in yield.

RESULTS WITH CUCUMBER

A limited survey of varieties of cucumber (*Cucumeris sativus* L.) indicated that young plants of the variety Early White Spine are very susceptible to the tobacco ring-spot virus. When inoculated with nondiluted native virus and cultured at warm temperatures, young plants are usually killed. It was found that mosaic mottling (Fig. 4) occurred in the chronic phase more regularly and at somewhat higher temperatures than in tobacco. This mosaic is readily confused with the mosaics induced by the cucumber-mosaic viruses, and with alfalfa mosaic virus 1A in cucumber (10).

During the summer of 1942, ten Early White Spine cucumber plants were grown in rich soil in large, deep, ground beds in a greenhouse without shade. Five of these plants were inoculated when they were small with tobacco ring-spot virus and the others left as controls.

FIG. 4. Leaves of Early White Spine cucumber. Healthy leaf upper left. Leaf at right of healthy leaf has primary lesions, other leaves show various expressions of secondary chlorosis and mosaic mottling during the chronic phase of the ring-spot virus infection. $\times \frac{1}{2}$.

All inoculated plants developed severe acute symptoms, and mosaic appeared at intervals on the new leaves throughout the season (Fig. 4). The control plants remained free from all diseases until at the end of the test when powdery mildew appeared. Throughout the season, the control plants were much more robust than the inoculated ones, and the latter gave no signs of improved vigor as they developed. Fruit was removed from the vines at intervals throughout the season, and at the end of the season the vines only were weighed.

TABLE 5.—*Data showing reduced yield caused by the tobacco ring-spot virus in Early White Spine cucumber plants grown in ground beds of fertile soil, in a greenhouse without shade, during the summer months, 1942*

Items	Healthy	Diseased
Average weight per plant without fruit	8.63 kg.	0.288 kg.
Average width of largest leaf per plant	22.13 cm.	13.34 cm.
Average length of longest petiole per plant	29.50 cm.	7.90 cm.
Average diameter of longest petiole per plant	8.33 mm.	5.50 mm.
Average length of longest runner per plant	3.45 m.	1.62 m.

The results of this test are given in table 5 and show that the ring-spot virus reduced the green weight of the plants about 97 per cent. This reduced weight was reflected in the smaller size of the leaves and in shorter length and smaller diameter of the runners.

RESULTS ON BEAN

In *Phaseolus vulgaris* L., var. Scotia, the ring-spot virus induced local lesions on the wiped primary leaves. At temperatures near 33° C., systemic infection was more rapid than at 22.5° C., and acute necrosis involved the secondary leaves, stem, and entire plant, causing death.

RESULTS ON PANSY

Pansy plants (*Viola tricolor,* var. Swiss Giant) were inoculated by all the methods used with tobacco and cucumber, and very active virus extracts were used as inoculum. The virus entered the plant readily when the leaves were wiped, but no signs of infection appeared on the wiped leaves. Occasionally vein clearing appeared in the new leaves, but no other signs appeared. The virus remained in the infected plants as long as they could be kept alive in the greenhouse. These tests have been repeated many times and always the same results were obtained. These results are at variance with those reported by Wingard (9).

DISCUSSION

The ring-spot disease and the yellow mosaic disease (5) in Samsun (Turkish) tobacco—each manifest an acute stage with pronounced symptoms and a chronic stage with reduced symptoms. Leaves well differentiated at the time of invasion show acute symptoms while leaves that are invaded at or near the time of their differentiation show chronic symptoms. However, the two diseases represent different levels of disease expressions. In the case of the yellow-mosaic disease the level of plant resistance was so low that in the chronic phase the reduced disease reactions were still very marked, whereas, with the ring-spot disease the level of resistance was so high in Samsun tobacco that ring-spot and mottling were not observed in the chronic diseased leaves. This reduced severity of the ring-spot disease was reflected in other varieties of tobacco, including genotypes that are highly resistant to tobacco mosaic. However, in some of these varieties and genotypes chlorosis and mosaic mottling appeared in certain chronic ring-spot diseased leaves at moderately low culture temperatures.

In pansy, resistance was so great that typical ring-spot symptoms were not apparent in any form, even though virus invaded the plant. However, in Early White Spine cucumber, the level of resistance to the ring-spot virus was sufficiently low to enable the expression of mosaic symptoms throughout most of the chronic phase.

Resistance to the ring-spot disease in tobacco was evidenced further by the fact that large amounts of virus and ideal culture conditions were neces-

sary to insure good acute reactions, especially in plants beyond the juvenile stage; and in such plants, the chronic phase appeared irregularly or not at all unless measures were taken to force large quantities of the virus into the very young apical tissues. It seems apparent that the level of virus synthesis is relatively low and that virus enters the meristematic tissue with difficulty. However, when virus does get into this tissue in sufficient quantity, perhaps into the apical cell, the subsequent young leaves manifest surprisingly few areas that are free from detectable virus. Since it appears that the characteristic masked or modified reaction of these leaves to the virus is predetermined by a high level of natural resistance in the very young tissues at the time of invasion, we are not at liberty to refer to these leaves as having recovered from the disease.

When these chronic diseased leaves were wiped with active ring-spot virus, they did not produce the primary acute reactions (necrotic lesions the ring spots) that resulted when healthy thrifty leaves were wiped with virus. The same results were obtained when yellow-mosaic virus was wiped on the chronic yellow-mosaic leaves on plants from the cross (*Nicotiana tabacum* × *Nicotiana longiflora* × *N. tabacum* (5). To expect these inoculated chronic-diseased leaves to react in the same manner as do inoculated healthy thrifty leaves would be contrary to logical expectations based on the evidence set forth in the studies on yellow mosaic (5), and to refer to this phenomenon as acquired immunity merely confuses the issue.

The growth-phase phenomena in the vertebrates differ so greatly from those in the seed plants, that the drawing of analogies (7) from either is extremely hazardous. The individual leaves of a tobacco plant possibly are comparable in a way to individual animals, but even this analogy is rather "far fetched." The practice of regarding the suppression of apparent symptoms in chronic diseases as acquired immunity, seems to accomplish nothing and it tends to obscure the more tangible lines of attack on the problem of disease expression and natural resistance in relation to developmental changes in the individual and to the gene mechanism.

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LATENT VIRUS OF DODDER AND ITS EFFECT ON SUGAR BEET AND OTHER PLANTS

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INTRODUCTION

In the spring of 1938 dodder, *Cuscuta californica* Choisy, was collected from a number of plants of a desert shrub known as California buckwheat, *Eriogonum fasciculatum* Benth., near Riverside, California, and placed on healthy sugar beets in the greenhouse preliminary to use in transmission experiments with the virus of sugar beet curly top. Within 10 to 15 days after the dodder began to grow on its new host, the sugar beet plants began to develop a type of chlorotic spotting or mottling that resembled somewhat that produced by some phases of common beet mosaic. However, the chlorotic areas were smaller, more uniform, and more intensely yellow than those commonly produced by beet mosaic and the leaves of affected plants were deformed. In further tests it was found that *C. californica*, parasitizing a number of desert species, was infected by a virus capable of producing these symptoms.

The virus is so unusual in its manner of occurrence and in the type of symptoms produced on sugar beet that it seemed worthwhile to make a more detailed study of its characteristics and host range. As will be shown, these studies revealed that a wide range of economic plants is susceptible to infection but the disease with its peculiar symptomology that would enable recognition has not been found in the field on any of these susceptible economic plants.

SYMPTOMS AND HOST RANGE

The host range of this virus, under desert conditions, has not been studied extensively. The virus was recovered only from dodder, *Cuscuta californica*, on which it produced no recognized symptoms of disease. No evidence of a diseased condition was found on any of the host plants on which infected dodder was growing. No virus was obtained from California buckwheat, *Eriogonum fasciculatum*, that had been parasitized by infected *Cuscuta californnica* and additional evidence obtained under greenhouse conditions indicates that this common host plant of dodder is immune from the virus. No other desert host plants of dodder were tested for presence of virus.

Using dodder as the agent of transfer, the virus was transmitted to several crop plants and to a number of common weeds. The symptoms of disease varied considerably on different host plants, but, since a type of mottling was more or less typical on most of the known susceptible species, the disease may be classified as a mosaic. The name Dodder latent mosaic is suggested for the disease. Representative symptoms are described on the following plants.

Sugar Beet (*Beta vulgaris L.*). First symptoms appeared usually on young leaves and consisted of more or less circular yellowish spots of various sizes. Spots were separate or confluent and in some instances affected leaves were almost completely yellow. Leaves produced later had less yellow color and the chlorotic spots were more widely scattered and larger, producing a mottled condition. Typically diseased leaves in early stages of attack are shown in figure 1.

FIG. 1. Early symptoms of disease on leaves of sugar beet plants inoculated by means of *Cuscuta californica*.

Leaves with the more severe symptoms were somewhat dwarfed, sometimes crinkled with irregular margins. Usually, new growth of the affected plant ceased to show symptoms after 6 to 8 leaves were produced. In rare cases this recovery from symptoms was not complete and faded areas were observed on leaves produced several weeks after infection. In most cases, however, plants completely recovered and no symptoms were evident on subsequent growth and the virus did not appear to interfere seriously with further development under greenhouse conditions.

Cantaloupe (*Cucumis melo L., var. Rockyford*). Leaves 2 to 3 cm. in diameter usually were the first to show symptoms, although yellow spots

appeared first on older leaves in some instances. On the younger leaves, chlorotic spots 2 to 3 mm., or more, long and about 1 mm. wide were produced on the veins. In places the growth of the veins was markedly retarded, resulting in a curled and twisted condition of the affected leaf. Chlorosis and necrosis increased as the leaf matured, and at maturity the leaf was about half normal size. Sometimes such leaves died prematurely. Two to four leaves showed these characteristics, but the severity of the symptoms decreased as more leaves were produced until a chronic condition was reached in which leaves, although somewhat reduced in size, were more or less normal in shape. Spotting of the type shown in figure 2 continued

FIG. 2. Chronic symptoms of disease on cantaloupe, var. Rockyford.

to be produced. The chlorotic spots were small but rather numerous and more conspicuous after the leaf was more than half grown. As the leaves became older, the chlorotic spots sometimes became necrotic in the center.

Plants inoculated in the greenhouse and transplanted to the field grew slowly and produced vines only about two-thirds as long as those on non-inoculated check plants. Leaves were much smaller than on normal plants and the melons were small and of poor quality. This evidence indicates that the latent virus of dodder is capable of causing a serious disease of cantaloupe if an efficient vector be available.

Potato (*Solanum tuberosum L.*). On potato plants of the White Rose variety, circular, dark, necrotic lesions 1 to 3 mm. in diameter were formed

on half-grown leaves 10 to 15 days after inoculation. The necrotic spots were sparse on some leaves, but more often they were abundant and more or less confluent. Some of the leaflets on which necrotic spots were numerous died before attaining maturity. In some cases, especially in the early stages of attack, the disease was more severe on one side of the leaf than on the other, resulting in the curving of the midrib and in death of some or all of the leaflets on the more severely affected side (Fig. 3, A).

Recovery from symptoms began to take place after 6 to 12 diseased leaves had been produced. In the initial stages of recovery necrosis became less marked; leaves were smaller, and mottling of the type shown in figure 3, B, involving chlorotic areas of various sizes and shapes with ill-defined margins,

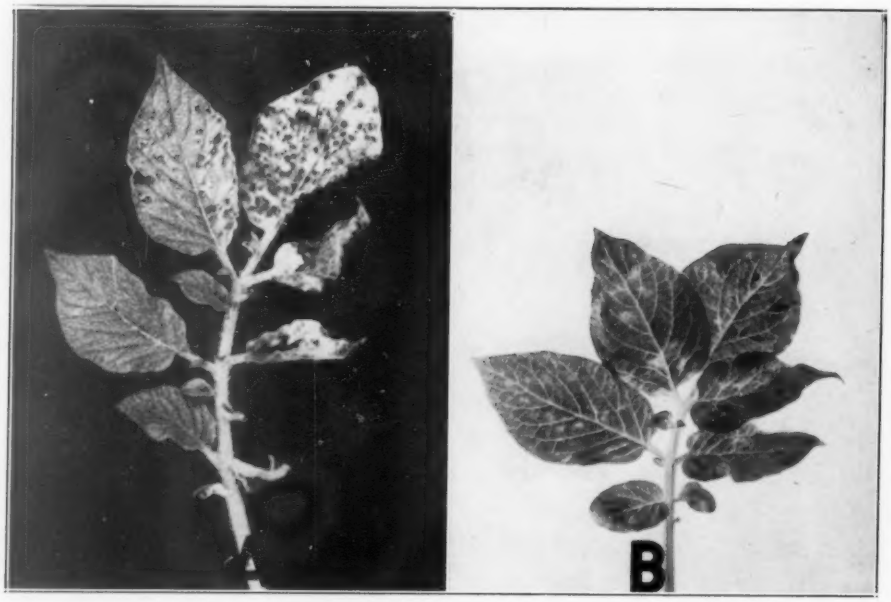

FIG. 3. Two phases of disease on leaves of potato, var. Early Rose. A, early stage of attack characterized by spotting, necrosis, and death of leaflets. B, later stage characterized by dwarfing and mottling.

was produced. As growth continued, mottling became less conspicuous until, after the production of 4 to 8 mottled leaves, growth again became normal.

Tomato (*Lycopersicon esculentum L.*). The younger leaves of tomato plants began to show water-soaked spots 7 to 14 days after inoculation. The spots turned brown within a few hours as the affected tissue dried. Spots were more or less circular and varied in size from 1 to 10 mm. in diameter. They were numerous and confluent or scarce. Usually not more than 3 leaves showed these necrotic spots. A very mild type of mottling was produced on the next 3 or 4 leaves. The terminal leaflets tended to twist, and often the lower surface was turned upward. Later growth appeared

normal. Diseased plants were not dwarfed either in the greenhouse or in the field and the yield of fruit seemed not to be decreased.

Celery (*Apium graveolens L.*). Very conspicuous primary symptoms were produced on the Golden Self Blanching variety of celery. The disease first made its appearance on leaves that were about one-third grown. In an area usually extending outward from the midrib into the leaflets, often chlorophyll was almost completely lacking and the affected parts were golden-yellow. The stage of development of the leaf when the virus entered evidently influenced extent and location of the chlorotic areas. Two to 4 leaves showed this type of chlorosis. The next 2 to 3 leaves were much

FIG. 4. Leaves of Golden Self Blanching celery showing (right to left) primary symptoms of disease and stages of recovery.

reduced in size and the leaflets were small, crinkled, and distinctly mottled. Following the production of these dwarfed and mottled leaves, recovery from symptoms was so complete that no evidence of abnormality was detected on any of the subsequent growth. A series of leaves of the Golden Self Blanching variety, showing transition from primary chlorosis through mottling to complete recovery, is shown in figure 4. Symptoms were somewhat less severe on the variety Utah.

Buckwheat (*Fagopyrum esculentum Moench*). Water-soaked spots, circular or more or less irregular in outline and varying in size, were produced on the young leaves of buckwheat plants 4 to 8 days after inoculation. These spots soon turned brown and the tissue became necrotic. This type of spot was restricted to 3 to 5 leaves. Later growth produced leaves somewhat reduced in size, mottled, and often rolled and deformed (Fig. 5). Under greenhouse conditions the yield of seeds was reduced considerably.

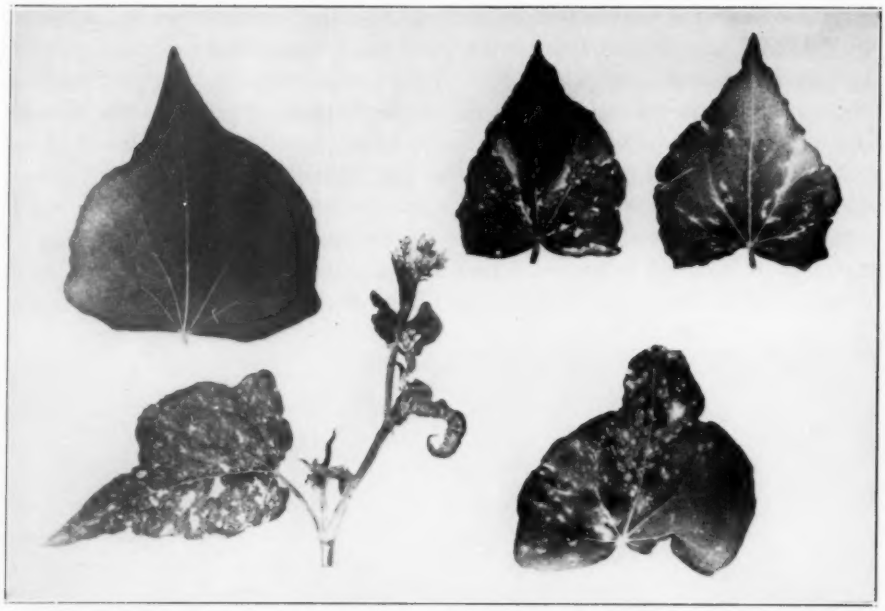

FIG. 5. Primary (separate leaves) and secondary (flowering shoot) symptoms on buckwheat.

Pokeweed (*Phytolacca americana L.*). Inoculations made by rubbing juice of infected plants over the surface of mature leaves of pokeweed resulted in the production of numerous local lesions usually on the third

FIG. 6. Typical symptoms of the disease on young pokeweed plants. The spots on the lower leaves are primary lesions produced by juice inoculation. Symptoms on the younger leaves are characteristic of those produced by systemic infection.

or fourth day following inoculation. The lesions appeared first as small, round, water-soaked areas about 1 mm. in diameter. They soon became necrotic and turned light-brown (Fig. 7, A). Subsequent development varied, depending on conditions. In most cases, as the leaves aged, reddish

FIG. 7. Two leaves of approximately the same age from pokeweed inoculated by the rubbing technique, using juice from recently infected pokeweed. Leaf A from a previously noninoculated plant and leaf B from a plant that had ceased to produce symptoms.

rings were formed around the necrotic areas and outside of these, broader rings of faded tissue were noted. In some cases on older leaves the primary lesions were limited to faded circular spots with no evident necrosis.

The first evidence of systemic infection consisted usually of a wilting of the tip of one of the smaller leaves, followed within a few hours by the pro-

duction of roundish water-soaked lesions on this and other young leaves. The lesions soon turned brown. Often, one of the smaller leaves was killed outright. Usually, necrosis was produced on 3 to 5 leaves (Fig. 6), but the severity of the diseased condition decreased rapidly in subsequent growth. After the plant passed from the necrotic stage, 3 to 5 leaves were formed that showed mild vein translucency and a type of mottling. This type of symptom was followed by complete or almost complete recovery from symptoms. In rare cases, leaves on recovered plants were smaller than normal, obscurely faded in the interveinal areas, and the veins of young leaves were slightly translucent.

The leaves produced after the plants recovered were immune from production of primary lesions following inoculation by the rubbing technique. Figure 7, A and B, shows typical results obtained from inoculation of leaves of the same size and age on noninfected (7, A) and recovered (7, B) plants. Leaves such as that shown in figure 7, A, that had only primary lesions were not completely immune from further infection. Virus concentration in symptomless parts of diseased plants was very low compared to that in parts with severe symptoms, as indicated by the relative numbers of primary lesions produced on pokeweed leaves by inoculations with juice from the two types of tissue.

Other Susceptible Plants. Tests were made on a number of other plants to determine susceptibility. The following species were found susceptible and to produce necrosis of varying degrees of severity followed by recovery from symptoms: water pimpernel (*Samolus floribundus* N.B.K.), knotweed (*Polygonum pennsylvanicum* L.), common plantain (*Plantago major* L.), sowbane (*Chenopodium murale* L.) and lamb's quarters (*C. album* L.). *Nicotiana palmeri* Gray also is susceptible, but symptoms are limited to a type of vein clearing from which the plant soon recovers.

By use of virus-free dodder, *Cuscuta californica,* virus was recovered from the following species of plants on which no symptoms were observed: tree tobacco (*Nicotiana glauca* Graham), *Nicotiana rustica* L., vars. English, Iowa, *pumila,* and *jamaicensis,* tobacco (*Nicotiana tabacum* L.) var. Turkish, and mustard (*Brassica incana* (L.) F. W. Schultz).

Nonreactive and Immune Plants. No symptoms were noted on the following species of plants, but no attempts were made to recover virus: *Nicotiana sylvestris* Speg. and Comes, Jimson weed (*Datura stramonium* L.), Tolguacha (*D. meteloides* DC.), *Solanum integrifolium* Poir., *S. gilo* Radd., pigweed (*Amaranthus retroflexus* L.), tumble-weed (*A. graecizans* L.), chickweed (*Stellaria media* L.), hawkweed (*Hieracium argutum* Nutt.), *Sisymbrium irio* L., yellow sweet clover (*Melilotus indica* (L.) All.), squash (*Cucurbita maxima* Duchesne) var. Hubbard, pumpkin (*Cucurbita pepo* L.) var. Jack o'Lantern, bean (*Phaseolus vulgaris* L.) vars. Stringless Green Pod and Kentucky Wonder, garden pea (*Pisum sativum* L.) var. Little Gem, stock (*Matthiola incana* R. Br.), carrot (*Daucus carota* L.), and parsnip (*Pastinaca sativa* L.).

The following species of plants appear to be immune, since no virus was recovered from plants by juice inoculation to pokeweed or by use of virus-free dodder: California buckwheat (*Eriogonum fasciculatum*), sunflower (*Helianthus annuus* L.), cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.), belladona (*Atropa belladonna* L.) and mullein (*Verbascum thapsus* L.).

TRANSMISSION OF THE DISEASE

Transmission by Use of Dodder. Three species of dodder, *Cuscuta californica, C. subinclusa* Dur. and Hilg., and *C. campestris* Yuncker, when established on diseased plants, are able to pick up the virus of this disease. All 3 species of dodder retain the virus indefinitely when growing on plants immune from infection, and evidently are hosts of the virus. When infected dodder is trained to a susceptible plant, infection follows in all cases so far as known, after the dodder becomes established. The efficiency of these 3 species of dodder in the transmission of the virus has aided greatly in the study of the host range of the virus.

Tests of Insects. Since it seems improbable that the virus would be maintained indefinitely in nature through transmission by dodder alone, it is logical to postulate that transmission by insects takes place. Search for an insect vector under field conditions has been limited to a few localities. No insect that would appear promising as a vector has been located on dodder or on any of the plants on which dodder was growing.

Under greenhouse conditions there has been no evidence of spread of the disease in compartments where red spiders, thrips, mealy bugs, or the sugar beet-root aphids were present. Extensive transmission tests, using the peach aphid, *Myzus persicae* Sulz., certain unidentified species of aphids, and the beet leaf hopper, *Eutettix tenellus* (Baker), have given negative results.

Absence of the disease, so far as known, from crop plants indicates that if an insect is involved in its spread, this vector is closely limited in its feeding to desert species, and thus has little opportunity to carry the virus to the crop plants shown to be susceptible to infection.

Transmission by Juice Inoculation. Juice inoculations were made by gently rubbing the surface of leaves or stems, on which a small amount of abrasive had been sprinkled, with a cloth pad saturated with inoculum. Infection was produced on 4 species of plants; pokeweed, *Chenopodium murale*, sugar beet, and *Cuscuta campestris*. Of these plants, pokeweed is by far the most susceptible to infection. Frequently, as many as 200 primary lesions per leaf are produced on inoculated plants. On smaller plants evidence of systemic infection soon follows, but on plants approaching the blossoming stage, often no further symptoms are produced. *Chenopodium murale* appears also to be rather susceptible to infection, since 34 of 48 inoculated plants became diseased. Sugar beet is more resistant. Only 26 of 120 inoculated plants became infected, and the largest number infected

in a single test was 9 plants infected of 20 inoculated. Rapidly growing plants appeared to be more susceptible to infection than slow growing plants. *Cuscuta campestris* probably is still more resistant to infection. Of 40 seedling plants inoculated and placed on pokeweed, only one gave evidence of being infected. Of 20 large plants inoculated, 3 became infected.

In addition to the 4 species of plants already mentioned in connection with juice inoculation, all other species of plants found susceptible to infection through inoculation by use of dodder were tested for susceptibility to infection by juice inoculation. Extensive tests were made especially with celery, cantaloupe, tomato, and buckwheat, all of which show marked symptoms of disease when infected through dodder. No evidence of infection was observed on any of the juice-inoculated plants of these species nor on any of the plants of other species inoculated, with the exceptions already noted. However, juice from diseased plants of celery, cantaloupe, tomato, and buckwheat produced primary lesions on pokeweed, showing that the virus was not quickly inactivated in the extracted juice of these plants. In

TABLE 1.—*Results of tests of seeds of susceptible species of plants for transmission of virus*

Plants from which seeds were taken for tests for presence of virus	Seeds tested	Seeds found to carry virus
	Number	*Number*
Cantaloupe var. Rockyford	882	0
Pokeweed	1073	0
Buckwheat	121	0
Cuscuta californica	163	0
Cuscuta campestris	1080	52

view of this evidence it seems probable that failure to obtain infection through juice inoculation in a number of known hosts of the virus is due to reactions in the injured cells of inoculated leaves that prevent increase or transport of the virus.

Transmission Through Seeds. Seeds were collected from infected plants of Rockyford cantaloupe, pokeweed, buckwheat, *Cuscuta campestris,* and *C. californica* for tests to determine whether the virus is seed-transmissible. With the exception of *C. californica,* the seeds were collected from the diseased plants soon after they were mature and planted within a few days to reduce the possibility of inactivation of the virus in stored seeds.

The seeds of cantaloupe, buckwheat, and pokeweed were planted in pots and the plants were held until they attained considerable size before they were discarded. The seeds of the two species of dodder were allowed to germinate in flats and the seedlings were transferred to small plants of pokeweed or sugar beet to determine whether they transmitted the virus to these test plants. The results of all tests are shown in table 1.

All of the seedlings from seeds of diseased cantaloupe, buckwheat, and pokeweed were healthy in appearance. Since neither cantaloupe nor buck-

wheat completely recovers from symptoms of the disease, it may be assumed that seedlings would show symptoms if infected. However, since pokeweed recovers from symptoms, it is by no means certain that infected seedlings from seeds of diseased plants would show symptoms. It was found. however, when the seedlings were inoculated with juice from recently infected pokeweed, that primary lesions were produced on all plants from the seeds tested. Since recovered pokeweed plants are immune from the production of primary lesions, this result is considered proof that the seedlings were virus-free.

Although no evidence was obtained that virus was transmitted through the seeds of cantaloupe, buckwheat, and pokeweed, the evidence is conclusive that it was transmitted through the seeds of *Cuscuta campestris*. In the tests made, 4.8 per cent of the seeds from infected plants carried virus. The results from seeds of *C. californica* are inconclusive, since the number of seeds tested is small and the seeds were more than a year old at the time of test. Unfortunately, neither *C. californica* nor *C. subinclusa* produces seeds readily in the greenhouse, and seed production on native host plants is sparse, making extensive tests for seed transmission of the virus rather difficult for these two species.

PROPERTIES OF THE VIRUS

All property studies of the latent virus of dodder were made with juice from recently infected pokeweed plants. The juice, after being subjected to the various treatments, was inoculated into leaves of rapidly growing pokeweed plants by gently rubbing the inoculum over the surface of the leaves with a saturated cloth pad after the leaves were sprinkled with a small amount of abrasive. Results were determined from counts of the primary lesions. Three to 5 plants were inoculated in each test, and lesions on 3 consecutive leaves of each plant were counted.

Thermal Inactivation Point. Juice was placed in slender thin-walled glass tubes of about 1 cc. capacity. The tubes were immersed for a 10-min. period in water held at the desired temperature. Upon removal they were immediately immersed in cold water. They were then opened and the content used to inoculate plants. The results of 3 tests shown in table 2 indicate that the temperature of inactivation lies between 56° and 60° C., much of the virus apparently being inactivated at 58° C.

Longevity in Vitro. Juice was placed in small flasks and kept at laboratory temperatures (about 24° C.) until used. Inoculations were made at intervals shown in table 2. Results indicate that the virus was inactivated rapidly in expressed juice of diseased pokeweed. After 48 hours no infection was obtained.

Tolerance of Dilution. Juice from pokeweed was diluted with distilled water and inoculated into pokeweed leaves. Dilutions and results of 3 tests are shown in table 2. These results show that number of infections dropped off rapidly in dilutions above 1–50, but a few lesions were obtained from dilu-

TABLE 2.—*Properties of dodder latent mosaic virus as determined by local-lesion counts following inoculation to pokeweed*

Thermal inactivation				Longevity *in vitro*				Tolerance of dilution			
Temperature (10-minute period)	Average number of local lesions per leaf in trial No.			Period aged at 20°–24° C.	Average number of local lesions per leaf in trial No.			Dilution	Average number of local lesions per leaf in trial No.		
	1	2	3		1	2	3		1	2	3
°C.	*No.*	*No.*	*No.*	*Hours*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*
Check[a]	19	30	48	Check[a]	42	23	28	Check[a]	28.4	36.0	46.3
54	7	23	44	2	27	26	34	1–10	28.8	27.7	40.0
56	3	16	21	6	10	25	58	1–50	20.6	18.1	20.3
58	0	1	0	12	4	6	31	1–100	8.2	2.3	9.2
60	0	0	0	24	2	1	2	1–500	.....	0.4	4.0
62	0	0	0	48	2	0	0	1–1000	1.2	0.4	3.3
				72	0	0	0	1–2000	.....	0.1	1.0
				94	0	0	0	1–3000	.....	0.1	0.3
								1–5000	0.0	0.0	0.0

[a] Fresh undiluted juice.

tions as high as 1–3000. Approximately the same results were obtained when juice from healthy pokeweed, instead of water, was used for making the dilutions.

Results of Other Tests of Properties. Other tests indicate that the virus retained activity in dried juice of pokeweed for less than 48 hours. No virus was recovered from dried leaves of pokeweed. The virus in juice of pokeweed passed readily through a one-half inch layer of Celite and through Berkefeld N and W filters with no detected decrease in concentration.

NAME AND DESCRIPTION OF THE VIRUS

The characteristics of the latent virus of dodder do not fully agree with those used in describing any of the 10 genera in the system of nomenclature and classification devised by Holmes.[1] On the basis of induced symptoms the virus agrees well with those placed by Holmes in the genus *Marmor,* but it does not conform to that part of the description of this genus that states regarding infected plants, "usually no recovery; if recovery occurs, no immunity to reinfection." However, in spite of the fact that host plants of the latent virus of dodder recover from symptoms and are immune from production of primary symptoms following a second inoculation, it seems best with this species to give major emphasis to induced symptoms and place it in the genus *Marmor,*[2] at least for the present. **Marmor secretum** sp. nov. (secretum, meaning hidden) is suggested as the Latin name of this virus. The following description of the species is presented.

[1] Holmes, F. O. Handbook of Pathogenic Viruses. Burgess Publishing Co. (Minneapolis, Minn.). 1939.

[2] It may be pointed out that the genus *Marmor* already includes at least 2 viruses (*Marmor rubi* and *M. persicae*) that cause primary symptoms from which there is a considerable degree of recovery. It seems doubtful whether in any case a plant has been shown to be immune from reinfection by any virus, although undoubtedly in some cases recovered plants are immune from production of primary symptoms following a second inoculation by the same or by a related virus.

Marmor secretum sp. nov. Attacks *Beta vulgaris* L., *Phytolacca americana* L., *Cucumis melo* L., *Fagopyrum esculentum* Moench, *Lycopersicon esculentum* L., *Solanum tuberosum* L., *Apium graveolens* L., and other plants. Typical symptoms consist of necrosis, chlorosis and mottling but all known susceptible species of plants partially or completely recover from symptoms of the disease. Produces primary lesions on inoculated leaves of pokeweed. Virus latent in *Cuscuta californica* Choisy, *C. subinclusa* Dur. and Hilg. and *C. campestris* Yuncker. Thermal inactivation point lies between 56° and 60° C. Inactivated *in vitro* at a temperature of about 24° C. in about 48 hours, withstands dilutions of 1–3000, retains activity in dried juice of pokeweed for less than 48 hours, and passes Berkefeld N and W filters.

No insect vector known. Transmissible to *Phytolacca americana, Beta vulgaris, Chenopodium murale* and *Cuscuta campestris* by juice inoculation. Also transmitted by *Cuscuta californica, C. campestris* and *C. subinclusa.* Transmitted through approximately 5 per cent of the seeds of *Cuscuta campestris* from diseased plants.

Descriptive Habitat. In *Cuscuta californica* on Box Springs grade 5 miles east of Riverside, California.

In the system of classification and nomenclature of Smith[3] the virus becomes *Cuscuta virus 1* and in the system of Fawcett[4] it should be designated *Cuscutavir secretum.*

DISCUSSION

At different times virus diseases have appeared in various parts of the world and swept rapidly over agricultural areas, sometimes causing enormous losses. Examples that may be cited are peach yellows in the northeastern part of the United States, curly top of sugar beets and other plants in western United States, and, more recently, mosaic of peach in the United States and yellow wilt[5] of sugar beet in Argentina. The source of virus for initial spread of these diseases to economic plants is more or less obscure in every case, but evidence indicates that the causal viruses were present in wild plants in the respective areas perhaps many years prior to their discovery on economic hosts.

The latent virus of dodder appears to present an example of a virus able to cause injury to several crop plants but has not yet encountered conditions that have permitted it to spread from wild to cultivated hosts and become established on the latter as a virus of economic importance. The virus probably has existed in native or introduced plants of Southern California for many years. But for the fact that it is transmitted readily to sugar beet by *Cuscuta californica,* probably it would have continued undiscovered so long as it remained restricted to its desert hosts.

Probably failure to find this disease on cultivated plants is due either to the absence of a vector able to carry it to cultivated plants or to the absence of a vector on such plants as are able to perpetuate the virus after it has made the transition from wild to cultivated hosts. So long as the present biological balance is maintained, it is reasonable to suppose that the virus will remain of no economic importance. However, the introduction of an insect able to function as an efficient vector of this virus might result in the sudden appearance and rapid spread of an entirely new virus disease on sugar beet, cantaloupe, celery, buckwheat, potato, and other crop plants.

[3] Smith, Kenneth M. A textbook of Plant Virus Diseases. P. Blakiston's Son and Co. (Philadelphia). 1937.

[4] Fawcett, H. S. Suggestions on plant virus nomenclature as exemplified by names of citrus viruses. Science, 92: 559–561. 1940.

[5] Yellow wilt is the name that in a forthcoming publication will be suggested for a virus disease that has caused serious damage to sugar beets from 1929 through 1941 in the Rio Negro valley of Argentina.

The presence of this virus concealed in desert plants, together with the evidence that a number of well-known viruses causing serious diseases of economic plants probably were derived from noncultivated species to which they caused little or no injury, suggests the possibility that innumerable other viruses may exist in wild plants of various parts of the world, many of which may have great potentialities for injury if the biological complex were so altered as to bring them into prominence on cultivated plants in their most destructive forms.

Although the disease caused by this virus is at present of no economic importance, the virus has certain characteristics that should be of interest to specialists. Among its interesting features is its failure to infect more than 4 of its 23 known host plants when introduced into plants by juice inoculation. Yet, one of its host plants (pokeweed) is so susceptible to infection by juice inoculation that numerous primary lesions are produced on inoculated leaves. As a rule, mosaic viruses that are transmissible by juice inoculation are transmissible by this technique to all or nearly all of their known host plants. However, with all such viruses the host range has been determined by juice inoculation supplemented in a number of instances by insect vectors and graftage. In the absence of dodder as an agent of transmission many of the plants now known to be susceptible to infection by the latent virus of dodder would be considered immune. The question arises, therefore, whether the latent virus of dodder is unusual in respect to the number of its host plants susceptible to infection by juice inoculation or whether the host range of many other mosaic viruses is imperfectly known because of the lack of, or failure to use, a method of inoculation that produces infection on all susceptible plants.

The fact that a mosaic virus exists that so far has produced infection only on a limited number of its host plants following juice inoculation, should offer encouragement for testing larger numbers of plant species for susceptibility to many viruses, such as those attacking members of the family Rosaceae, that are now considered nontransmissible by juice inoculation.

The latent virus of dodder is of interest also because of the initial severity of the disease that it produced on several of its host plants and the degree of recovery from symptoms that these plants attain in later stages of growth. Because of these characteristics and the accuracy with which relative virus concentrations may be estimated by the primary-lesion method using pokeweed, this virus may prove to be a valuable addition to the group of viruses now available for use in certain types of studies of recovery and immunological phenomena in plants.

SUMMARY

A new virus was discovered in dodder, *Cuscuta californica*, in the vicinity of Riverside, California, when dodder was removed from certain desert plants and established on sugar beet.

This virus produces mottling or necrosis, or both, on sugar beet, cantaloupe, tomato, potato, celery, pokeweed, *Chenopodium murale,* and other plants. All of the susceptible plants partly or completely recover from the more severe symptoms. Cantaloupe and buckwheat have shown the least recovery. *Cuscuta californica, S. subinclusa, C. campestris, Brassica incana* and certain species and varieties of *Nicotiana* are symptomless carriers of the virus.

No insect vector is known. However, the virus is transmissible by the 3 species of dodder, *Cuscuta californica, C. subinclusa,* and *C. campestris.* Transmission is readily obtained by juice inoculation to pokeweed on which the virus produces numerous primary lesions. It is transmissible less readily to *Chenopodium murale,* sugar beet, and *Cuscuta campestris.* No infection resulted from juice inoculation to any other species of plant. The virus was transmitted through slightly less than 5 per cent of the seeds of *C. campestris,* but not through seeds of cantaloupe, pokeweed, or buckwheat.

The thermal inactivation point of the virus lies between 56° and 60° C. Infection was obtained from juice of pokeweed diluted 1–3000. Activity was lost in juice of pokeweed in about 48 hours, in dried juice in less than 48 hours and no virus was obtained from dried parts of infected pokeweed plants. The virus passed Berkefeld N and W filters readily.

Dodder Latent Mosaic is suggested as the common name of the disease and the **Marmor secretum** sp. nov. is suggested as the Latin name of the virus.

Although this virus has not been observed to occur naturally on any crop plant, it is considered that with the introduction of an efficient vector the virus might be capable of producing a destructive disease on cantaloupe and buckwheat and a disease of lesser importance on sugar beet, potato, celery, and perhaps other plants.

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A SERIOUS STORAGE ROT OF CELERY CAUSED BY THE FUNGUS ANSATOSPORA MACROSPORA N. GEN.

A. G. NEWHALL

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INTRODUCTION

In the muckland celery-growing areas of New York State a number of storage rots occur. Those caused by *Sclerotinia sclerotiorum, Botrytis cinerea, Erwinia carotovora,* and, occasionally, *Phoma apiicola* are already well known, but within the past decade another fungus probably has caused more loss to stored celery than any of the others. During this time it has been mistaken for *Phoma apiicola,* as described by Bennett (2), but recently its true identity as a fungus close to the genus *Cercospora* has been pointed out by the author (5).

RANGE AND ECONOMIC IMPORTANCE

Specimens and complaints have come chiefly from 6 cold storages in Wayne County, New York. Losses have been sustained, however, in a number of other counties, including Chautauqua, Niagara, Monroe, and Onondaga, and the trouble is known also in Ontario, Canada. Although these facts indicate a fairly widespread occurrence, nevertheless losses tend to be confined to the late-grown celery from certain particular farms or fields.

The disease seems not to have been described on celery, and there is a paucity of references to it in the literature on market diseases. Bratley and Wiant (1), in a summary report on the diseases of fruits and vegetables, found by them on the New York market during the last 3 months of 1939, state that, "Approximately 70 per cent of the stalks in 90 crates of upper New York State celery received in mid-December was found affected with a black crown rot. Although the symptoms were indistinguishable from those of Phoma root rot (*Phoma apiicola*) none of the isolates have developed fruiting bodies." C. W. Bennett, who described the Phoma root rot in this country in 1921, on examining a culture of this new fungus wrote the writer that it was definitely not *Phoma apiicola.*

VARIETIES ATTACKED

A number of standard types of celery have been grown 2 years on muckland farms, where the black crown rot was prevalent. None have been found immune. Late in October 1 or 2 crates of each variety were placed in commercial cold storage and held until January, when final examinations were made. In both years the celery looked healthy and free from rot at the time of harvest. In the first year it was tall and well-grown, but in the second it was stunted by the exceptionally dry season. In neither season could any evidence of crown rot be found before 7 weeks had elapsed in storage.

In the first test of 11 varieties from 15 to 50 per cent of the total of 1393 plants became affected in 10 weeks of storage. In the second test of 15 varieties from 9 to 65 per cent developed the rot in 11 weeks' time. There was no difference in susceptibility between green and yellow varieties. Easy Bleaching and Tall Golden Self Blanching were less affected than others in both tests, while Pascal and Golden Plume were among the

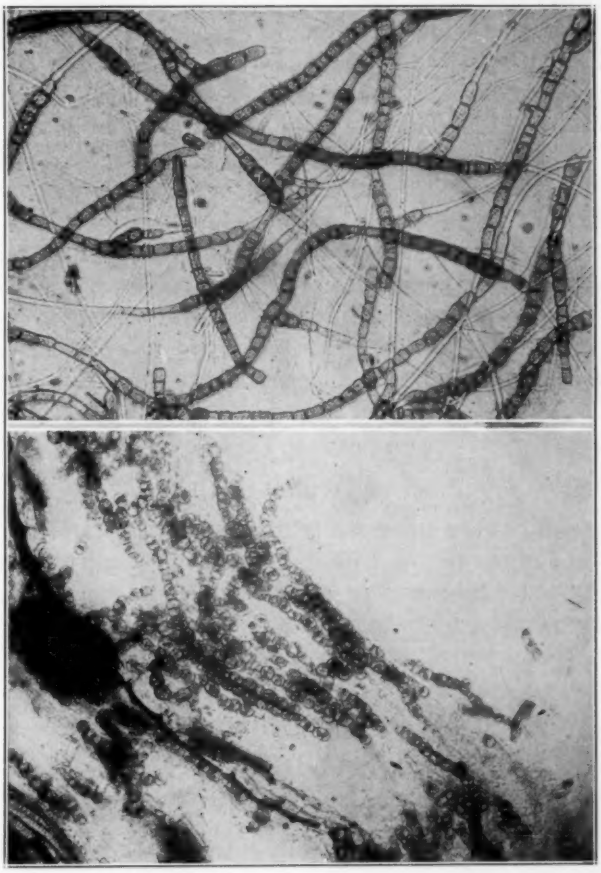

FIG. 1. Upper: An 8-day-old culture of *Ansatospora macrospora* on potato-dextrose agar, showing variable size of mycelium, the beginning of the torose condition and of deep coloration of older hyphae. ×85. Lower: Old mycelium of *Phoma apiicola* in celery tissue, showing its similarity to *Ansatospora*. ×85.

most severely attacked. The varieties tested were Easy Bleaching, Columbia, Utah, Tall Golden Self Blanching, Fordhook, White Queen, Old Golden, Golden Phenomenal, Giant Pascal, Meisches Special, Golden Plume, Abbott & Cobbs No. 14, White Plume, French Golden, Early Fortune, and Meisches Improved Golden. While the percentages of infection varied, the differences are not considered to be significant.

SYMPTOMATOLOGY

Diseased plants have rarely been found in the field. After celery is placed in cold storage, symptoms appear in 7 to 8 weeks, which usually means at sometime between November 25 and December 31. The first indication of infection is the appearance of a light ochraceous-tawny[1] colored lesion about 5 mm. deep somewhere on the butt end of the celery. As the brown rot advances further into the butt, the mycelium within becomes strongly torose and very dark colored (Fig. 1). This causes the color of the lesion to change from dull hazel to a dark olivaceous, which later becomes greenish slate-black[1] (Fig. 2, B and C). It is this ultimate greenish-black color that distinguishes the disease from other storage rots. The surface of the lesions often present a somewhat shiny appearance but it is not a particularly wet rot if unaccompanied by secondary bacterial pathogens. Later in the storage period, the characteristic dark lesions may be found at almost any point on the outer leaf stalks, indicating inoculation through wounds initiated possibly at harvest time. Conidia may be borne on the black lesions at room temperatures. Humidity is thought to be a very important factor in their production. Sometimes a distinctly red color may be imparted to the celery tissue near the outer margin of rapidly advancing lesions.

THE CAUSAL AGENT

Proof of Its Pathogenicity

Celery butts have been successfully inoculated with pure cultures of the black crown-rot fungus obtained by tissue plantings, and the fungus again has been recovered. Single-spore cultures obtained both from naturally infected celery plants and from agar cultures have proved pathogenic to celery stalks in cold storage and to celery seedlings grown under sterile conditions on agar, and to celery leaves. On the latter, lesions similar to early blight are produced. On their lower surface sporulation occurs if humidity is favorable (Fig. 3, A).

Cultural Characters and Morphology

The mycelium of the black crown-rot fungus is distinctive. It is not likely to be confused with any of the other common celery storage rotting fungi except perhaps *Phoma apiicola* (Fig. 1). At 18° C. on corn-meal agar a 3-inch Petri dish may be completely covered in 8 days, indicating a growth rate 3 or 4 times faster than that of either *Phoma apiicola* or *Cercospora apii*. Young hyphae are hyaline, mostly 3 μ in diameter, and are not conspicuously branching. After 3 to 5 days' growth on corn-meal or potato-dextrose agar at room temperatures older hyphae become 6 to 9 μ wide and soon develop some color varying between red, brown, and bluish green depending on the substrate. The cell walls thicken and the mycelium becomes strongly torose. The individual chlamydospore-like cells are at

[1] Ridgway, R. Color standards and color nomenclature, 45 pp. and 53 col. plates. (Washington, D. C.) 1912.

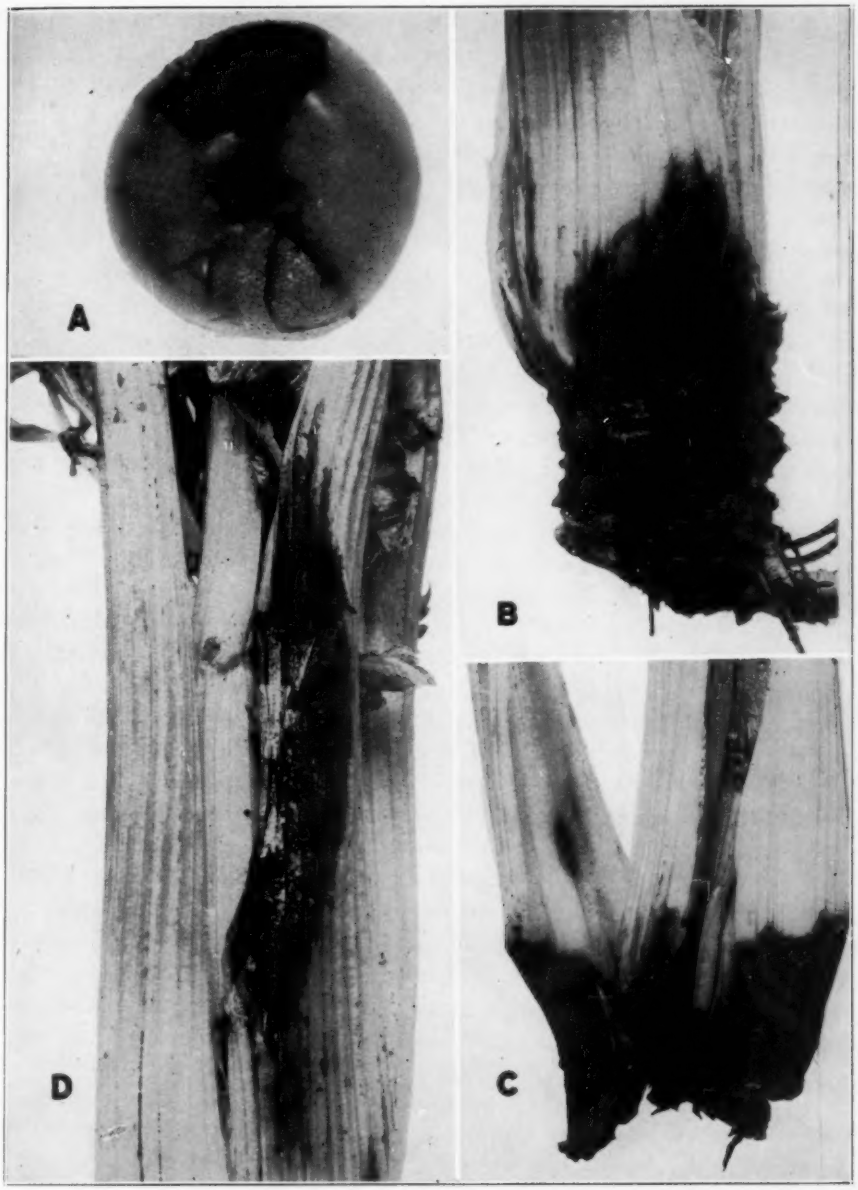

FIG. 2. A. An apple eighteen days after inoculation on the lower side with an actively growing culture of *Cercospora apii* and on the upper side with a culture of *Ansatospora macrospora* from celery. On carrots the same results were obtained. B. Typical crown rot symptoms, after 10 weeks in cold storage, involving entire crown and advancing up the leaf stalks. C. Late stage on celery as sometimes received on the market with butt end completely gone. D. Lesion on stalk near first joint where a bruise was probably sustained at harvest time.

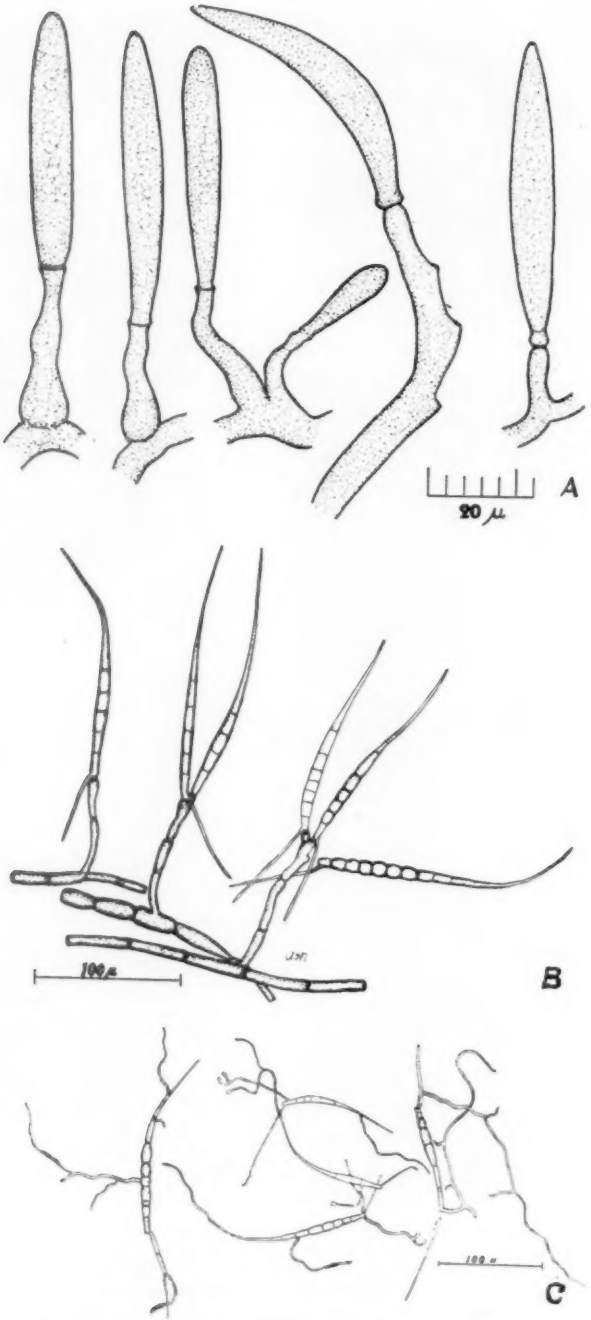

FIG. 3. A. Young conidiophores bearing conidia, from the lower side of a celery leaf spot 10 days after inoculation with a pure culture of *Ansatospora* from celery. B. Mature conidia borne on subhyaline conidiophores, from corn-meal agar culture. C. Conidia, germinating after 12 hours. A and C drawn with aid of camera lucida.

first 9 to 12 μ in diameter and later become 15 to 20 μ in 2 to 3 weeks with a few reaching 30 microns. They become ovate to spherical and very dark, olivaceous-brown to dark bluish or olivaceous-green. The same process occurs in the tissue of the host, which accounts for the distinctive color changes taking place there as the rot advances. On plain corn-meal agar, 6-day-old, rapidly growing cultures at 16° C. often develop a ring of bright red outside of the dark green central region. This has been seen also on diseased celery plants brought from cold storage to a more favorable temperature for rapid fungus growth.

Aerial mycelium is white and abundant, though not dense, on 2 per cent potato-dextrose or corn-meal agars. Alternate periods of desiccation and high humidity promote sporulation.[2] The subhyaline conidia are borne sparingly on potato-dextrose agar, more abundantly on corn-meal agar, after 30 days. More conidia and less mycelium are produced on potato and corn-meal agars low in sugars and much brighter red color occurs about the young hyphae.

The most abundant conidial production was obtained by employing the method mentioned by Westerdijk and van Luijk (6). After the fungus mat had nearly reached the sides of a Petri dish the agar was quickly inverted in the same dish. In a few days at room temperature sporulation occurred over the freshly exposed surface of the agar. Conidiophores arise singly or in clumps from young subhyaline mycelium. They are hyaline to subhyaline and bear 1 to several, falcate, hyaline to subhyaline conidia, 120 to 210 μ long (mean 160 μ), by 6 to 11 μ wide (mean 8.84 μ) (Fig. 3). Nearly half the spore consists of a long, tapering, whip-like beak, and a prominent, distinguishing feature on most spores, as they near maturity is a delicate, slender sword-like appendage 2 μ by 90 μ, which protrudes from the side of the basal cell at an obtuse angle. Westerdijk and van Luijk found some with 2 such appendages, but the author found more that seemed to lack them entirely. These conidia differ from *Cercospora apii* in being borne on shorter, nearly hyaline conidiophores, in being obclavate rather than acicular, in being twice the width, and in having the peculiar appendage just described. Germination occurs from any of the cells including the beak and tip of the appendage (Fig. 3, C). In culture they may germinate *in situ* and may anastomose freely.

Many efforts to induce the development of a sexual stage in pure culture and on celery stalks have been made but to no avail. The fungus, therefore, must remain at present as one of the fungi imperfecti falling in the Scolecosporae of the Dematiaceae in the key of Clements and Shear (3).

Identity of Crown Rot Fungus

In 1924, Osterwalder (4) described a leaf spot of pansy (*Viola tricolor*) caused by a fungus that seems to agree with the one described here. He called it *Cercospora macrospora*, although some of the conidia were not

[2] L. L. Hill, a graduate student, first found conidia during a routine classroom exercise in comparing the growth of several fungi isolated from celery.

strictly hyaline. In his illustrations the sword-like appendage is clearly visible, although he makes no mention of it. Specimens of a similar leaf spot of *Viola*, collected September 3, 1934, by G. F. Gravatt near Wrangell, Alaska, and sent to C. Chupp at Cornell, have been examined and found to agree with Osterwalder's description, except that conidiophores were not hyaline but brown. Conidia appeared identical with spores from the celery black crown rot fungus. Furthermore, in 1941, a culture that proved to be identical with the celery fungus was sent to Dr. Chupp by C. M. Tompkins of the University of California. This, it was stated, was obtained from a leaf spot of *Viola* on which the fungus was very destructive.

A few months after Osterwalder's description of his Viola disease in Germany, Westerdijk and van Luijk (6) described a fungus attacking caraway (*Carum carvi*) in Holland. The description fits that of *Cercospora macrospora* and the fungus causing black crown rot of celery. They mention the presence of peculiar appendages on the conidia and admit difficulty in assigning the fungus to any known genus because of them and of the variable color of the spores, which, on aging, do not remain hyaline. In naming their fungus *Cercospora cari* n. sp., they suggest that it probably deserves generic rank.

Since the writer has found the black crown-rot fungus from celery capable of causing lesions on caraway and on leaves of *Viola*, and the fungus from California has proved pathogenic to celery, there seems little doubt but that the cause of black crown rot is the same as the fungus causing leaf spot of pansy and anthracnose of caraway. If it were to be retained in the genus *Cercospora*, the name given it by Osterwalder, *Cercospora macrospora* Osterw., would stand. But, in a proposed monograph of the genus *Cercospora* by C. Chupp, no place exists for any forms having conidia with appendages of any kind. At his suggestion, therefore, a new genus, *Ansatospora*[3] is proposed for those fungi resembling *Cercospora* and *Cercosporella*, but whose conidia possess one or more appendages, cilia, or secondary conidia. The name proposed for the black crown-rot fungus is *Ansatospora macrospora* (Osterwalder) Newhall. Synonyms would include *Cercospora macrospora* Osterw. and *C. cari* (Westerdijk and van Luijk). The description follows:

Mycelium variable; at first hyaline, 3 μ in diameter, becoming darker, wider, and torose with age. Color becomes very dark olivaceous, while diameter increases to 15 or 18 μ. Conidiophores single or in small clusters, hyaline at first, 1–3-celled, geniculately bearing, terminally or laterally, 1 to several conidia. Conidia obclavate, falcate, hyaline to subhyaline, 120–210 μ long by 6 to 10 μ wide. Widest point a little below middle, 4 to 11 septa, truncate base, terminal, whip-like, beak 30 to 110 μ long. A straight to slightly curved whip-like appendage (rarely 2 of them) from 30 to 105 μ long by 2 μ wide, protrudes from basal cell at approximately a 45° angle with the main axis. Habitat, *Viola tricolor*, *Carum carvi* and *Apium graveolans*, causing leaf blight of pansies, anthracnose of caraway, and a crown and stalk rot of celery in cold storage.

Host Range

No attempt to explore the host range has been made beyond that deemed necessary to establish the identity of the crown rot pathogen. Small lesions

[3] Ansata = Latin for handle, hence spores with a handle-like appendage.

were obtained on the petioles of potted caraway and parsley plants grown from seed in the greenhouse. Similar lesions were obtained by inoculating leaves of *Viola tricolor*. When apples and carrots were inoculated with *Ansatospora* in comparison with *Cercospora apii*, only the former was able to infect (Fig. 2, A). This pronounced ability to cause rotting of vegetables and fruit at low temperatures is considered additional reason for separating *Ansatospora* from the typically leaf spotting Cercosporae. The suspicion that many other hosts occur has been confirmed by recent unpublished work of Tompkins and Hansen in California.[4]

EFFECT OF ENVIRONMENTAL FACTORS ON GROWTH AND PATHOGENICITY OF ANSATOSPORA MACROSPORA

Ansatospora macrospora, like *Phoma apiicola* in pure culture, has an optimum temperature close to 17° C. On corn-meal agar growth takes place between 0° and 27° C. but not at 31°. This low optimum and ability to grow at low temperatures partly explain the destructive action of this fungus in cold-storage celery.

Good growth of *Ansatospora macrospora* has been obtained on potato-dextrose agar adjusted to a pH ranging from 3.35 to 7.32. The optimum seems to lie very near the neutral point.

The fungus was found to endure desiccation in celery leaf stalks dried at room temperature for a period of at least 7 weeks, when the last of this tissue was pulverized, moistened with sterile tap water, and used to inoculate the butt ends of healthy celery placed in cold storage at 2–3° C. At the end of 11 weeks one of the 21 inoculated plants showed typical crown-rot lesions in which the fungus was clearly observed under the microscope. Three of the 4 plants inoculated with an actively growing agar culture of the pathogen became infected, and the 7 checks remained free from infection. It is not known whether conidia were present as part of the inoculum or not. Old dry agar cultures have been revived after 18 months of slow desiccation at room temperatures.

Some difficulty was experienced in early attempts to prove pathogenicity of *Ansatospora* for celery butts until adequate means were devised to maintain humidity in the experimental cold-storage chamber. An indication of the importance of this factor of humidity is afforded by the following experiment.

Three crates of celery from a supposedly infested field were brought to Ithaca and stored in a cold chamber at 32° F., where the humidity was approximately 60 per cent. A similar lot of 3 crates, placed in commercial cold storage, where the relative humidity was over 90 per cent, was kept under observation for over 2 months. At Ithaca 2, 4, and 14 per cent, respectively, of crown rot developed, while in the commercial cold storage 28, 42, and 47 per cent developed in the same period of time. Unfortunately, at the lower humidity the celery loses its turgidity, shrivels, and becomes

[4] The writer acknowledges their helpful criticism in the preparation of this manuscript.

practically worthless. Manipulation of the humidity factor, therefore, does not offer a hopeful approach to the question of control of crown rot.

CONTROL OF CROWN ROT

1. Fungicidal Dips at Harvest

On the assumption that crown-rot infections occur primarily at harvest time, perhaps from soil inoculum, a number of attempts were made to protect the butt ends of celery with fungicides as soon as cutting and trimming operations were completed in the field. Several fields were selected known to grow a crop that later developed crown rot in storage. Approximately 80 plants taken at random were trimmed and dipped for a few seconds in

TABLE 1.—*Results of celery butt dipping experiments for control of crown rot, after 9 to 11 weeks in cold storage*

Fungicide (strength)	Number rotting per crate					
	Farm					Mean
	A	B	C	D	E	
1. Lignosan[a] (1 part in 400 of water)	0	.....	.....	0	0	0.0
2. Ethyl mercury phosphate (2½ per cent) (1 part in 400)	2	0	2	0	0	0.8
3. Wet check—water	5	0	2	1	4	2.4
4. Dry check—no treatment	5	1	2	3	5	3.3
5. Copper carbonate (18 per cent) (1 part in 50)	8	0	6	3	0	3.4
6. Palustrex B[a] (6 tsp. in 2 gal.) = 1 per cent	4	1	8	1	0	3.5
7. Waxlac No. 69 + 1 per cent Palustrex B	.....	2	7	2	6	4.2
8. Waxlac No. 69[a]	7	0	.....	1	.....	3.6
9. Waxlac No. 69 + sol. of Hexamethalene tetramine ¼ per cent	.....	0	11	.....	3	4.6
10. Cuprous oxide suspension (1 part in 50)	2	0	8	8	2	4.0
11. Formaldehyde (1 part in 200)	1	4	12	6	6	6.4
12. Copper sulphate (1 part in 30)	2	24	17	29	11	16.6
13. Copper sulphate + starch (9 oz. each in 2 gal.)	6	23	29	.....	.....	19.0

[a] *Lignosan*, a powder containing ethyl mercury chloride 4.3 per cent; *Palustrex B*, a water-soluble pine oil containing 15 per cent copper-resinate; *Waxlac*, an emulsion containing carnauba wax, used in protecting nursery stock in storage.

a metal pan 3 inches deep containing one of the fungicides. The celery was then packed in crates holding 70 to 80 plants and placed in cold storage at 0° ± 1° C. where it was examined at weekly intervals beginning 7 weeks later. There were 11 liquid fungicidal treatments, besides one or two dry ones not reported on. The experiment was repeated on 5 farms, with slight modifications and included a total of between 4,500 and 5,000 plants. The materials used and the number of rotted plants per crate found, 9 to 11 weeks later, are given in table 1.

There were striking differences in appearance of the celery treated with the different fungicides. Those plants dipped in the organic mercurials were dry and clean looking and practically free from butt rot. Those dipped in the copper sulphate solutions were badly russeted, shrunken, and the prey of numerous molds. Several crates of celery treated with the carnauba

wax emulsion (Waxlac) developed green surface mold, which markedly lowered the appearance of the celery. The crates dipped in water were no worse than the dry checks, but, under field conditions, the 1-minute dip in water served to keep the butts wet only a few minutes longer than the so-called dry checks.

On 2 farms the butts were cut very short; in fact, flush with the crown. On 2 others a 1- or 2-inch stub of a tap root was left on, but seemed not to influence the subsequent amount of rot. It was concluded that, except for organic mercurials, the fungicides as used in these experiments were not able to prevent butt rotting. In similar tests dry sulphur and solutions of boric acid were tried and also found ineffectual.

To obtain light on the poisonous properties of celery receiving the mercurial butt treatments just described, half a bushel of butts were cut off and fed to guinea pigs and laboratory rats over a period of 2 weeks. This work was done in the laboratories of the Departments of Animal Nutrition and of Veterinary Pathology and Bacteriology of the New York State Colleges of Agriculture and Veterinary, respectively. During the feeding period supplementary foods were given these animals. They gained in weight and showed no outward signs of ill effects from the celery. At the end of the period after the animals had eaten more than their own weight of treated butts, the rats were autopsied. No internal symptoms of toxicity of any kind were found.[5] It is readily admitted, being negative data, that this is insufficient evidence to establish the safety of the use of a mercurial salt on such a crop of celery. The writer *emphatically does not recommend it,* even for butt ends that normally would be discarded. The apparent control obtained with such fungicides lends encouragement to the belief that infection occurs at the time of harvest and to the hope that some day an effective but safe treatment may be found.

2. Rotation of Crops

At present it appears that a rotation in which celery occupies the land once in 3 years is not long enough to eliminate this disease entirely. Celery growers are reluctant to adopt a longer one, however. The fact that carrot roots are also susceptible raises a question about the wisdom of growing this crop in the rotation on suspected land. Growers have obtained the most relief by growing only their early crops of celery on their suspected land, reserving the fields known to be disease-free for the late crop, which is likely to be stored. Crops harvested by September 5, usually are consumed long before crown rot can develop.

If celery from infested or suspected land is put in cold storage, it behooves the owner to begin examinations at weekly intervals after the 7th week. When a few soft butts can be detected by poking the fingers up between the bottom slats of the crates, the crop should be marketed within the next few days. It is when this precaution has been neglected that the greatest losses have been sustained, losses that have reached 100 per cent.

[5] Thanks are due Miss Gladys Sperling for her kind cooperation in making these tests.

COMPARISON OF BLACK CROWN ROT WITH OTHER STORAGE DISEASES OF CELERY

Since the black crown rot now occurs in important celery-growing areas of New York and Ontario, Canada, and since it may appear in other localities, a review of the essential characters by which it may be distinguished from the other principal storage rots is in order.

Mention has been made of the fact that symptoms are rarely found at harvest time and of the marked color changes that characterize symptoms of this disease, beginning with a light tawny brown and ending with its dark greenish black, sometimes bordered by a distinct red at the margin of rapidly advancing lesions. None of the other common celery storage rots caused by *Botrytis*, *Sclerotinia*, *Phoma*, or *Erwinia* goes through such a color range. Phoma root-rot lesions, with which crown rot may be confused, may advance up the leaf stalks but are a dark brown rather than a greenish black color; older crown infections present a rough, dry, cracked and distinctively scurfy surface. Numerous dark pycnidia often may be found by the aid of a hand lens on young as well as old affected plants. Under the microscope, lesions due to *Phoma*, like those of *Ansatospora*, are found to be filled with a dark torose mycelium nearly as large as that of *Ansatospora* (Fig. 1). But in culture *Phoma apiicola* grows only about one fourth as rapidly as *Ansatospora* and undergoes much less marked color changes. In New York the Phoma rot is an infrequent storage disease. It has been found occurring on seedlings and young plants as a severe root rot in the spring or fall. It has not persisted in the same field year after year, as the black crown rot has.

A summary of the distinguishing characteristics of the 5 principal storage rots is presented in condensed form as in table 2.

DISCUSSION

The origin of *Ansatospora macrospora* in New York State is unknown. The fact that Westerdijk and van Luijk found that it could be carried by the seed of caraway is suggestive. This host has long been grown in herb gardens in this State, and has escaped in places where it may now be found growing wild on waste land. Search for diseased "native" caraway plants has not been fruitful so far. Neither has the disease been found on pansies or other violas, although these are commonly grown. Other hosts may be found and their discovery may yet throw light on the origin and prevalence of the black crown rot of celery.

Since *Cercospora apii* is known to be carried very commonly in celery seed, it would not be surprising to find *Ansatospora* disseminated in the same manner.

Attention is drawn to the similarity between the leaf-spot symptoms of *Ansatospora macrospora* and *Cercospora apii*. Both fungi cause a brown necrotic lesion on the under side of which the conidia are borne. The possibility of *Ansatospora* being disseminated by air-borne conidia cannot be denied.

TABLE 2.—*Some distinguishing characters of five storage rots of celery*

Pathogen	*Ansatospora*	*Botrytis*	*Sclerotinia*	*Phoma*	*Erwinia*
Name	Black crown rot	Grey mold soft rot	Watery pink rot	Phoma root rot	Bacterial (slimy) soft rot
Occurrence	Storage and transit	Storage and transit	Some late field, mostly storage and transit	Mostly field, spring and fall, some storage and transit	Field, storage and transit
Gross host symptoms	Firm to soft, olivaceous-black crown and stalk rot	Soft to watery, tan to buff, crown and stalk rot	Watery, pink to creamy, crown and stalk rot	Scurfy, cracked firm, dark brown root and crown rot	Very mushy, wet rot, light-tan color and some odor at times
Signs of pathogen	None, though few conidia may show under microscope	Dense, drab-grey mold on older lesions. Black thin sclerotia *embedded in substrate*	Cottony white to pinkish surface mycelium. Black, thick sclerotia *on the mycelial surface growth*	Minute pycnidia with black papillae seen on older lesions with hand lens	Occasional soft greyish-tan, buttery glistening blisters filled with bacteria
Some cultural characters of pathogen	Mycelium variable in size from 3 to 20 μ, and in color from hyaline through *red, brown, green* to *olivaceous-black* on corn-meal agar or steamed rice. Older *mycelium strongly torose.* Covers Petri dish *in 8 days* at optimum temp. of 17° C.	Mycelium mostly 10 to 15 μ, hyaline to faintly colored, *conidia usually abundant,* giving drab color to culture, followed by *sclerotia closely embedded* in substrate. Optimum temp. 23° C.	Mycelium like *Botrytis* hyaline, *no conidia, sclerotia* more meaty and *occur superficially in the mycelial mat.* Optimum temp. 18° C.	Mycelium scanty variable in size and *becoming torose* like *Ansatospora* but *not as broad or as bright colored and takes 25 days* to cover Petri dish at optimum temp. of 16° C. *Pycnidia form in 5 to 10 days* on corn-meal agar	Typical bacterial colonies on agar

There is no evidence that the disease is picked up after the celery is brought to the cold-storage plants. There is little if any spread from one lot of celery to another, piled next to it, in the same room.

SUMMARY

The term "black crown rot" is proposed for a serious storage disease of the butt ends of celery causing considerable loss to growers and produce men in Western New York and Canada.

The disease is readily distinguishable from the other common storage diseases caused by *Botrytis*, *Sclerotinia*, *Phoma*, and *Erwinia*, by its ultimate black color, by its lack of aerial mycelium, or sclerotia, or pycnidia, or slime, by the presence of very dark olivaceous torose mycelium within the tissue and later of conidia sparsely borne on the surface of the black lesions.

The fungus causing this disease is thought to be the one previously described by Osterwalder as the cause of a pansy leaf spot, named *Cercospora macrospora* by him. Its identity with the fungus causing anthracnose of caraway, described and named *Cercospora cari* by Westerdijk and van Luijk also is established. Its name is changed to **Ansatospora macrospora** (Osterw.) n. gen. chiefly on the basis of the long, prominent appendage protruding from the basal cell of the conidium. A full description of the fungus is given.

Temperature limits for growth in culture were close to 0° and 31° C. with the optimum near 17° C. Good growth on agar occurred at hydrogen ion concentrations between pH 3.35 and 7.32 with the optimum near neutrality.

The fungus is capable of attacking leaves and stems of celery, stems and fruits of caraway, stems of parsley, and of rotting apples and carrots.

Circumstantial evidence indicates that black crown rot may be soilborne. A 3-year rotation was not long enough to eliminate it from some fields.

Control by dipping the butt ends of freshly harvested celery in a number of different fungicides met with little success. Such treatment is not advised.

Since the disease rarely can be found until celery has been in storage for at least 7 weeks, growers have been successful in reducing losses by using fields, thought to be infested, for growing only their early crop of celery, which is never stored. Celery from infested fields if placed in cold storage should be carefully watched after 8 or 9 weeks, and, if symptoms appear, should be promptly marketed to avoid loss.

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THE RELATION OF SEED QUALITY TO THE DEVELOPMENT OF SMUT IN OATS[1]

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Satisfactory epidemics of seedling-infecting smuts are obtainable only by inoculating seed of a susceptible variety with viable spores of the pathogen under initial and prevalent favorable environmental conditions.

The spores must make contact with the caryopsis. For oats there are 2 methods: (1) Hull removal and dusting of caryopsis with chlamydospores; (2) Replacing by vacuum pump the air beneath the hull with a water suspension of chlamydospores. Both methods successfully place the inoculum upon the caryopsis, and epidemics are readily obtainable by either method. Many (1, 2, 3, 4, 6) have studied environmental factors governing infection. It is generally recognized that relatively dry soil at approximately 20° C. is most favorable to smut infection of oats, although moderate fluctuation of either factor does not prevent infection. Lods and Coulson (5) found that seedlings from small seeds of one variety were more susceptible to smut than those from large seed. The writer has confirmed their findings; and now reports quantitative results of more detailed experiments on plants from various seed lots of oats.

MATERIALS AND METHODS

Five varieties of oats were included in the experiments. Several seed lots were obtained of each variety (Tables 1, 2, 3), differing in place of origin and year of production. In 1940, one collection of *Ustilago levis* (Kell. and Sw.) Magn. and one of *U. avenae* (Pers.) Jen. were used, while in 1941 only *U. levis* was tested. With the exceptions stated in the text, seed was inoculated by the partial-vacuum method, a chlamydospore suspension of 0.5 g. smut in 100 cc. sterile water being used to inoculate 25 g. seed. In the few cases when the dry-spore method of inoculation was used, 0.5 g. of chlamydospores was used for 25 g. seed. Each seed lot was replicated 5 times in field experiments, 5 g. of seed being planted per 8-ft. row. Smut percentage was determined by head count, with an average of 200 heads per 8-ft. row, although older seed lots gave as few as 60 heads per row. The experiments on smut were made in the field at University Farm, St. Paul, Minnesota, in 1940 and 1941, while experiments on the relative germination rates of seed lots were made in the greenhouse at University Farm during the same years.

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Variability in Amount of Smut in Oat Varieties

In 1940, seed of Anthony C.I. 2143, Gopher C.I. 2027, Rusota C.I. 2343, and Iogold C.I. 2329 was inoculated with one collection, each, of *Ustilago levis* and of *U. avenae,* while only Anthony and Gopher and one collection of *Ustilago levis* were used in 1941 (Table 1). Similar results were obtained in both years. Plants from different seed lots of the same variety differ greatly in their reaction to smut. The seed lot of Anthony grown at St. Paul in 1939 produced plants that were only slightly smutted, while plants of the same variety from seed grown at St. Paul in 1936 were most heavily

TABLE 1.—*Percentage smut produced on plants from different seed lots of oats by Ustilago levis and U. avenae*[a]

Origin of seed lot		Variety, year of test, species of smut, and percentage of smutted heads									
		Anthony			Gopher			Rusota		Iogold	
Where grown	When grown	1940		1941	1940		1941	1940		1940	
		avenae	levis	levis	avenae	levis	levis	avenae	levis	avenae	levis
St. Paul	1935	26	46	61	.....	.....	.....	.....	.....	10	21
	1936	40	72	89	20	50	51	20	14	26	20
	1938	14	38	53	2	11	33	13	4	8	10
	1939	2	14	18	1	2	5	3	1	6	6
	1940	.....	.....	49	.....	.....	.....	.....	.....	.....	.....
Waseca	1935	.....	.....	.....	2	15	13	7	7	.....	.....
	1938	28	54	.....	1	19	23	6	3	2	10
	1939	16	43	80	1	9	17	5	3	1	3
Crookston	1937	16	45	65	1	16	14	11	5	3	10
	1938	10	43	73	1	7	9	2	2	1	3
	1939	10	37	82	1	7	12	4	3	1	3
Duluth	1938	13	42	75	1	6	8	7	4	0	0
	1939	3	22	54	0	2	4	1	1	0	1

[a] The writer wishes to express his deep appreciation of the kindness of H. K. Wilson, Division of Agronomy, University Farm, St. Paul, Minn., for supplying the seed lots used in this test.

attacked. However, some seed lots that gave plants only slightly smutted in 1940 produced more heavily smutted plants in 1941. Plants from seed lots grown at Waseca, Crookston, and Duluth, Minnesota, in 1939 were attacked more severely in 1941 than in 1940. Also, plants from the 1940 seed lot from St. Paul were significantly more severely attacked than plants from the 1939 St. Paul seed lot.

The varieties used in the above test were obtained from the supplies at the various branch experiment stations of the University of Minnesota and were supplied initially from the Agricultural Experiment Station at St. Paul. Because of the possibility that the lots obtained from the station in different years differed somewhat genetically, seed of Anthony and Black Mesdag oats produced at St. Paul in 1939 were sent to several stations in

the United States and Canada for increase. These 1940 seed lots[2] were inoculated with *Ustilago levis* and planted in St. Paul in 1941. Significant differences in the amount of smut were found in plants from the different seed lots. The percentage of smutted heads in Anthony ranged from 49 from the Texas lot to 83 from the Kansas lot, while in Black Mesdag the maximum amount of smut was 60 per cent from the Washington seed lot and only 7 per cent from the Texas seed (Table 2). However, there was only enough Texas seed to plant 2 replicates, and the plant sample may have been too small. However, the 28 per cent smut in plants from the South Carolina seed lot is significantly lower than the 60 per cent in plants from the Washington lot. Thus from one original seed lot of an oat variety, seed lots can be produced in different localities that give rise to plants differing widely in their susceptibility to smut.

TABLE 2.—*Percentage smut produced by Ustilago levis at University Farm, St. Paul, Minn., in 1941 on plants from seed lots of oats grown in different localities in 1940*

Origin of seed	Variety and percentage of smutted heads	
	Anthony	Black Mesdag
Texas	49	7[a]
West Virginia	50	52
South Carolina	52	28
Ohio	66	54
Alberta	75	43
Washington	81	60
Montana	82	46
Kansas	83	38

[a] Average of 2 replicates only.

Additional evidence to indicate that the differences between plants from various seed lots were not due to genetic differences was obtained from the testing of seed grown in 1940 from St. Paul seed lots of earlier years. In 1940, 4 seed lots of Anthony oats that were produced at St. Paul in 1935, 1936, 1938, and 1939 respectively, were inoculated and sown in the field at St. Paul. There were decided differences in the amount of smut in plants from the different lots (Table 1). In 1940 seed was obtained from these 4 lots of Anthony and inoculated with *Ustilago levis* in 1941, but there were only relatively slight differences in the percentage of smut in the plantings, the amount of smut varying from 30 to 45 per cent (Table 3). Seed produced in 1940 from the 1939 seed lot gave rise to plants more heavily smutted than plants from the 1939 seed lot, while seed produced in 1940 from the 1936 seed lot gave plants less heavily smutted than plants from the 1936 seed lot. Thus the susceptibility of plants from the 4 seed lots of Anthony appeared to result from the action of environment on the crop that produced the seed and not from genetic differences between the seed lots.

[2] The writer wishes to express his deep appreciation for the collaboration of the following men who in 1940 assisted in increasing the seed for this experiment: J. G. Leach, Morgantown, West Virginia; C. C. Allison, Columbus, Ohio; I. M. Atkins, Denton, Texas; T. W. Graham, Florence, South Carolina; L. E. Tyner, Edmonton, Alberta; C. S. Holton, Pullman, Washington; A. M. Schlehuber, formerly of Bozeman, Montana, and E. D. Hansing, Manhattan, Kansas.

TABLE 3.—*The amount of smut produced in 1941 on plants from four seed lots of Anthony oats grown in 1940 at St. Paul, Minn., the plantings having different amounts of smut in 1940*

Seed lots grown in 1940 from St. Paul seed lots produced in the years given below	Per cent smutted heads
1935	30
1936	45
1938	44
1939	31

A further test on the variability of oats to smut was available from seed grown at St. Paul in 1940. Seed of Anthony, Gopher, and Black Mesdag was sown at intervals during the spring of 1940 and seed from the plants grown that year was available. In addition, seedlings of these varieties had been germinated in the greenhouse and then transplanted to the field at intervals during the spring of 1940; and seed from these transplants also was available. The seed lots so obtained were inoculated with *Ustilago levis* and planted in the field in 1941. Only small differences in the amount of smut in plantings of these seed lots were found (Table 4).

Great variation in susceptibility to smut exists within plants from different seed lots of a variety of oats. Naturally, it is important in testing oat varieties to races of the oat smuts to recognize the possibility that variation in susceptibility of different lots may affect the relative susceptibility of varieties in different years. The average amounts of smut produced on Anthony oats by 6 collections of *Ustilago levis* at St. Paul, in 1939, 1940, 1941, and 1942, are 52, 8, 24, and 29 per cent, respectively, and with 5 collections of *U. avenae* in the same years, 48, 12, 16, and 30 per cent, respectively. The seed used in 1939 was from the 1938 seed lot, that of 1940 from the 1939 seed lot and, similarly, in 1941 and 1942, the seed from the preceding harvest was used. It was shown (see Table 1) that plants from the 1939 Anthony seed lot were smutted less heavily than those from 1938 and 1940 lots. It

TABLE 4.—*Percentage smut produced by Ustilago levis at University Farm, St. Paul, in 1941 on plants from seed lots of oats grown at different times in 1940 at University Farm, St. Paul, Minn.*

Seed lot derived in 1940 from		Variety and percentage of smutted heads					
		Anthony		Gopher		Black Mesdag	
Seed planted on	Seedlings transplanted[a] on	Seeded	Transplanted	Seeded	Transplanted	Seeded	Transplanted
April 24	April 23	61	36	9	14	40	37
May 3	May 2	35	41	14	11	47	45
May 9	May 7	49	66	12	7	43	41
May 18	May 16	64	46	9	7	38	41
May 25	May 25	33	29	9	11	36	32
June 10	June 5	.....	.....	4	5	25	17

[a] Seedlings transplanted to field from greenhouse when 5 to 8 days old.

would appear, therefore, that the seed lot of Anthony used in the 1940 smut tests was of great importance in causing the low amount of smut obtained in that year. Environmental conditions were not unfavorable to smut development in 1940, since Iogold was attacked at least as heavily as in most other years, and, indeed, in 1940, was more susceptible than Anthony.

It is important also to know, if possible, the reasons for the variation in susceptibility of different seed lots to smut. Two possibilities exist to explain these variations: (1) When inoculated with chlamydospores by the partial-vacuum method, different amounts of inoculum may get between the hulls and the caryopsis in different seed lots; (2) That the seed lots produce plants differing in physiologic potentialities that would determine the extent of smut attack.

Physical and Physiological Factors Influencing Smut Infection in Anthony Oats

Successful smut infection requires that the inoculum be in contact with the caryopsis. The seed lots used in the tests already described differed in

TABLE 5.—*The effect of dehulling seeds on and the relation of seed weight and volume to susceptibility to smut of plants from seed lots of Anthony oats*

Year seed lot produced at St. Paul, Minn.	Percentage smutted heads		Weight in g. of 1000 seeds		Volume in cc. of 1000 seeds	
	Normal	Dehulled	Normal	Dehulled	Normal	Dehulled
1935	61	74	15.1	8.8	50	24
1936	89	90	15.8	8.6	48	22
1938	53	75	14.1	9.6	46	22
1939	19	61	24.3	20.4	57	34
1940	49	77	17.5	13.4	49	26

relative weights and plumpness, and, hence, possibly would differ in the ease whereby chlamydospores could be placed adjacent to the caryopsis. The partial-vacuum method for smut inoculation replaces air between the hulls and the caryopsis by a water suspension of chlamydospores. Hence, differences in the degree of adhesion of the hulls to the caryopsis would affect the deposition of inoculum on the caryopsis. By inoculating the naked caryopsis, a standard quantity of chlamydospores could be applied.

Five seed lots of Anthony oats grown at St. Paul, in 1935, 1936, 1938, 1939, and 1940, were dehulled and the naked caryopsis inoculated by dusting with 0.5 g. dry chlamydospores per 25 g. seed, and the inoculated seeds planted in the field in 1941. The dehulled seed consistently produced more smutted plants than that inoculated by the partial-vacuum method, the greatest increase being in plants from the 1939 seed lot, in which 19 per cent smut was produced with the partial-vacuum method, while 61 per cent developed from dehulled seed inoculated with dry chlamydospodes (Table 5). However, the 1939 seed lot was still significantly less severely attacked than the 1936 lot, which had 90 per cent smut. Thus plants from seed lots

of one variety showed significant differences in response to smut when the seed was inoculated by the partial-vacuum method, while relatively slight differences were found following inoculation of the caryopsis with dry chlamydospores. It is probable then that the degree of adhesion of the hulls to the caryopsis is important in determining the spore load carried by a seed lot inoculated by the partial-vacuum method.

Since the opportunity for infection was similar for all seed lots inoculated with dry spores, the relatively smaller amount of smut in plants from the 1939 seed lot must be due to the physiologic response of this seed. Data on the weight and volume of the 5 seed lots were obtained (Table 5). Seeds of the 1939 lot were heavier and larger than seed lots of other years. It

TABLE 6.—*Relative germination rate of 3 seed lots of Anthony oats in steamed and field soil in greenhouse*

Treatment of seed		Seed lot	Field soil					Steamed soil				
			Stand[a] 12 days after planting	Per cent total stand, days after planting				Stand[a] 12 days after planting	Per cent total stand, days after planting			
				4	5	6	7		4	5	6	7
No inoculation	Normal seed	1938	62	0	60	81	86	104	2	47	72	76
		1939	210	21	82	92	94	204	22	77	89	95
		1940	129	11	55	72	79	168	7	44	61	81
	Seeds without hull	1938	40	8	56	80	83	92	14	62	73	87
		1939	145	57	85	91	95	208	49	81	89	93
		1940	82	17	51	73	81	156	26	55	71	82
Smut-inoculated	Normal seed	1938	87	6	62	85	90	111	6	36	70	83
		1939	196	24	81	90	91	196	14	79	91	93
		1940	133	8	50	68	83	177	4	54	69	79
	Seeds without hull	1938	14	0	36	57	71	82	10	65	88	94
		1939	126	41	77	84	88	194	55	82	89	92
		1940	43	7	49	65	86	172	23	66	83	88

[a] Total of 5 replicates, of 50 seeds each.

appears that plants from heavier seed tend to be less severely smutted than plants from lighter seed, this agreeing with the observations of Lods and Coulson (5).

In a preliminary report (9), the writer stated that the 1939 seed lot of Anthony oats germinated more rapidly than the other seed lots tested and, because of this more rapid germination, less time was available during which the seedlings could be infected. Germination tests on 3 seed lots of Anthony oats were made, the experiments being made in the greenhouse in field soil and steamed soil (equal parts of field soil and sand). Comparisons were made between normal and dehulled seed, and between inoculated and non-inoculated seed. Normal seed was inoculated by the partial vacuum method, while the dehulled seed was dusted with chlamydospores. Five replicates of 50 seeds each for each soil type and seed treatment were used, the seeds being planted uniformly at ½ in. depth.

In both steamed and field soil, the 1938 lot gave the poorest stand, while the 1939 lot was best. Little difference in stand appeared in steamed soil between normal and dehulled seed, whether inoculated or not, but in field soil dehulling of the seed greatly reduced the stand, a further reduction in stand occurring with smut-inoculated dehulled seed (Table 6). Tapke (7) has reported similar results. The relative germination rate of the 3 seed lots was similar on both soils and with all seed treatments, the 1939 lot in every case germinating more rapidly than the 1938 and 1940 lots (Table 6). In fresh weight and height, the seedlings of the 1939 lot consistently were heavier and taller than the 1938 and 1940 lots (Table 7). In general the

TABLE 7.—*Relative height and weight of seedlings from 3 seed lots of Anthony oats in steamed and field soil in greenhouse*

Treatment of seed		Seed lot	Seedling height in mm. and weight in g.[a]					
			Field soil			Steamed soil		
			Height in mm., days after planting		Average seedling test wt.	Height in mm., days after planting		Average seedling test wt.
			6	11		6	11	
					g.			*g.*
No inoculation	Normal seed	1938	20	58	0.141	23	58	0.140
		1939	37	87	0.240	29	73	0.227
		1940	25	73	0.207	25	72	0.171
	Seeds without hull	1938	21	50	0.138	24	37	0.150
		1939	42	74	0.246	37	79	0.224
		1940	22	53	0.187	31	74	0.180
Smut-inoculated	Normal seed	1938	21	53	0.114	21	56	0.132
		1939	35	72	0.267	32	70	0.236
		1940	29	70	0.201	27	72	0.164
	Seeds without hull	1938	13	32	0.177	23	56	0.156
		1939	33	70	0.245	36	77	0.201
		1940	19	46	0.170	33	73	0.158

[a] Seedling height measured from ground level to tip of uppermost leaf.

average seedling weight in field soil was somewhat greater than in steamed soil, and this difference may be due to the elimination of weak seedlings that would be more susceptible to attack of seedling-blight organisms.

It is apparent that plants of the 1939 seed lots as indicated by rapidity of germination, percentage germination of the seed and weight and height of seedlings were more vigorous than plants from the other two seed lots tested. Also, more vigorous seedlings tend to be less liable to infection by smut than weaker seedlings of the same variety. If vigorous seedlings tend to resist infection by smut more than weaker seedlings, then the 1939 lot, after storage for a period of years, should give rise to plants that are more readily infected. Tests run in 1942, with the 1938, 1939, 1940, and 1941 seed lots inoculated by the partial-vacuum method gave the following percentages of smut, respectively, 43, 25, 38, and 18. The differences in the

amount of smut in plants from the 1938, 1939, and 1940 seed lots planted in 1942 are therefore considerably less than the differences that were found in 1941 or between plants of the 1938 and 1939 seed lots in 1940 (Table 1). The relatively greater susceptibility of plants from the 1939 lot in 1942 in comparison with those from the 1938 and 1940 lots confirms somewhat the hypothesis given above, namely, that as seeds of a susceptible variety become older and become less vigorous in their germination capacity, they will produce plants more susceptible to smut.

DISCUSSION

During the past 10 years, field tests have been made at St. Paul to determine the pathogenicity of the collections of the oat smuts on several varieties of oats. It has been shown that some of the collections varied in pathogenicity on one variety in successive years and the variation was governed by the variety on which the smut inoculum had been increased (8). Obviously, different conditions at and immediately following planting will influence the degree of infection; therefore, the amount of smut on a susceptible variety should vary from year to year, depending on the occurrence of conditions suitable for infection. But the amount of smut formed also will be governed by the characters of the seed lot used. It has been shown that the seed-lot characteristics are of great significance in affording variation to attack by smut and that these characteristics may be as important as the environment under which the plants are grown in successive years.

The determination of physiologic races in the oat smuts, especially under field conditions, is complicated because plants from seed lots of the same variety of oats may be attacked by smut to different degrees. Any attempt to replicate in successive years the conditions of any one year, can approach success only if recognition be given to (a) the genetic stability of the smut collection, (b) the genetic purity of the host variety, (c) the requirement, as far as possible, of similar conditions of the soil in regard to temperature and moisture, at planting and for a few days thereafter, and (d) the utilization in successive years of seed lots producing plants that are attacked by smut to the same degree. The first two requirements are fairly easily solved; for smut collections, when purified by increase on one variety, tend to remain fairly stable, and standard varieties of oats show little variation. It is, of course, not possible always to obtain soil conditions favorable for infection. Nor can the variation of seed lots be readily controlled.

It has been shown that plants from seed lots of one variety are attacked more uniformly by smut when inoculation of the seed is accomplished by removal of the hull and dusting of the naked caryopsis, than when the partial-vacuum method of smut inoculation is employed. However, dehulling of seed is a slow and expensive procedure, and where many varieties are to be tested with several collections of smut, the more rapid partial-vacuum method is preferred. It is practical, however, to use one seed lot for a period of years for, although the viability of the seed will tend to decrease

with age, good stands can still be obtained with 5-year-old seed. If sufficient seed from one crop year can be obtained and used in determining physiologic races of smut for a 5-year-period, then yearly differences in the amount of smut produced will be reduced. The writer is of the opinion that the use of one seed lot of each variety for a period of up to five years will assist materially in interpreting the data obtained, since the variation in the amount of smut on plants from different seed lots will be reduced.

SUMMARY

Plants from seed lots of Anthony, Gopher, Rusota, Iogold, and Black Mesdag oats were tested for resistance to *Ustilago levis* and *U. avenae*.

Great variation in the amount of smut was found in plants from seed lots within one variety, the variation being as great as may be found between certain varieties.

It was shown that variation in the amount of smut occurred with plants from seed lots of one variety grown in different years in the same locality, and between plants from seed lots of the same variety grown in one year in widely different localities.

The differences in the amount of smut apparently are not due to genetic differences in the plants but to modifications in the seed resulting from the particular environmental conditions under which the seed was produced.

When seed lots were dehulled and inoculated by dusting dry chlamydospores on the naked caryopsis, smaller, although still significant, differences in the amount of smut were found than when the partial vacuum method of inoculation was used.

One seed lot of Anthony oats, when inoculated by the partial-vacuum method, produced plants with only 19 per cent smut, while 61 per cent smut was produced when the caryopsis was dusted with chlamydospores. In plants of other seed lots, only slight differences in the amount of smut resulted from the use of the two methods of inoculation. It is concluded that the relative tightness of the hulls surrounding the caryopsis is responsible for the difference in the amount of smut on plants from different seed lots.

The seed lot of Anthony oats that produced fewest smutted plants germinated more rapidly and gave larger seedlings than other lots of Anthony. It is concluded that the more vigorous seedlings of Anthony oats are less frequently attacked by smut than weaker seedlings.

It is considered that recognition should be given to differences in seed lots as a partial explanation of the variation occurring in smut tests made over a series of years. It is suggested that more uniform results in the determination of physiologic races of smut would be obtained by using seed from one harvest for a succeeding period of years rather than obtaining new seed lots each year.

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RECOVERY FROM CURLY TOP IN THE TOMATO IN RELATION TO STRAINS OF THE VIRUS[1]

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All varieties of *Lycopersicon esculentum* Mill. and of *L. pimpinellifolium* (Jusl.) Mill. are, so far as the writers are aware, susceptible to curly top. Under field conditions in the semiarid western United States, plants of certain varieties of tomato commonly recover from severe stages of the curly-top disease. The new growth of recovered plants shows mild curly-top symptoms, but if recovery be persistent and growth continue, the plants tend to become more nearly normal, so that late in the season they sometimes show only very slight evidence of the disease. This type of recovery has been observed only rarely in cultivated varieties of tomato, but in a primitive race of *L. esculentum* collected in Mexico and referred to as "Guasave A,"[2] a large proportion of the plants showed this reaction. Such plants, or cuttings grown from them, continued to harbor the virus of curly top, even though the plants were nearly normal in aspect. It was shown further that these plants were usually highly resistant to injury from reinoculation by means of the beet leaf hopper, *Eutettix tenellus* (Baker), the vector of the curly-top virus. Such plants are, therefore, considered to have attained a condition of acquired immunity similar to that developed in tobacco and tomato and described by Wallace.[3,4,5]

Each year for a period of several years, at the University of California Citrus Experiment Station, the writers have inoculated plants of Guasave A and its derivatives with the virus of curly top, and a detailed study of the reactions of these plants has been made. The reactions of this tomato race to curly top were not consistent from year to year, the time of the beginning of recovery, the proportion of plants recovering, and the degree and persistance of recovery, being highly variable in different years. For example, of 15 Guasave-A seedlings inoculated June 2, 1939, 14 plants recovered, 6 of the plants making a good, rapid recovery and becoming nearly normal in appearance. On the other hand, there was no case of recovery among 12 plants of Guasave A inoculated June 21, 1940. Two families, one of 27 plants and the other of 38 plants, derived from the backcross (Break o' Day × Guasave A) × Guasave A, were inoculated on June 6, 1939, and June 21, 1940, respectively. In the 1939 test, 16 plants (59 per cent) recovered; of

[1] Paper No. 496, University of California Citrus Experiment Station, Riverside, California.

[2] Lesley, J. W., and J. M. Wallace. Acquired tolerance to curly top in the tomato. Phytopath. 28: 548–553. 1938.

[3] Wallace, J. M. Evidence of passive immunization of tobacco, *Nicotiana tabacum*, from the virus of curly top. Phytopath. 30: 673–679. 1940.

[4] Wallace, J. M. Evidence of passive immunization of plants from curly top. (Abstract) Phytopath. 30: 26. 1940.

[5] Wallace, J. M. Virus strains in relation to acquired immunity from curly top in tomato. (Abstract) Phytopath. 32: 18. 1942.

these plants, 4 recovered early and made nearly normal growth. In 1940, only 6 plants (16 per cent) derived from the same backcross recovered, and these did not recover to a degree equaling that of the plants of the 1939 test. A similar variability was shown in an F_3 family from Riverside × Guasave A. Fifteen plants were inoculated in each year, 1939 and 1940. In 1939, 7 plants recovered, 2 of which developed into vigorous, almost symptomless plants. In contrast, only 2 plants of the 1940 group showed evidence of recovery, and both of these died after making a feeble recovery. By the chi-square test with Yates's correction, the odds against the occurrence, in random sampling, of the differences observed in these 3 pairs of values are about 24 to 1.

The influence of climatic conditions on the initiation and persistence of recovery is probably considerable. For the most part, tomato plants, even of varieties that have a tendency to recover, experience a severe initial shock following infection with curly-top virus. High temperatures speed the development of the disease and increase its severity. Under conditions extremely favorable for disease development, it appears that plants are often injured so severely and rapidly that there is not sufficient time for the reaction leading to recovery to take place and become effective. In the course of these investigations, however, the climatic conditions at the time of inoculation of the plants and during the periods following inoculation, probably did not differ sufficiently from year to year to account for all the observed variability with respect to recovery of the plants.

The discovery that differences in virus strains play an important role in the variability of acquired immunity from curly top in the tomato,[6] suggested that the variations in recovery in Guasave A and its derivatives, from year to year, might result partly from variations in the composition of the virus used for inoculation in these tests. The variations in recovery cited above occurred in tests made prior to 1941, when no special attention was given to the strains of virus used for inoculation of the plants. The plants were inoculated by means of leaf hoppers from many different stock colonies, some of which carried single, known strains of the virus, while others carried one or more unidentified strains.

In 1941 and 1942, tests with Guasave-A plants and known strains of the curly-top virus were, therefore, undertaken. It is the purpose of this paper to report the results obtained.

EXPERIMENTAL RESULTS

In 1941, 5 different strains of curly-top virus were used singly for inoculation of Guasave-A tomato plants. Virus strains 3, 5, 9, 58,[7] and 75 were selected because of their known virulence on cultivated tomato varieties.

[6] See footnote 5.

[7] Beet leaf hoppers carrying the individual strains of curly-top virus were supplied by N. J. Giddings, Senior Plant Pathologist, Division of Sugar Plant Investigations, U. S. Department of Agriculture, Riverside, California. Strain numbers above 10 have been tentatively assigned by him to strains under study but not yet given permanent numbers.

Five groups of 35 plants each were used. Each group was inoculated with 1 of the 5 virus strains, each plant having 15 leaf hoppers confined to a portion of the plant by a sleeve-type celluloid cage. The insects were caged on the plants on June 11, 1941, and the cages and surviving insects were removed 1 week later. General observations were made on the different groups of plants during the developmental period of curly top, and records were made of the incidence and severity of disease. The reactions of the plants in the various groups and the influence of virus strains on recovery are brought out in table 1. At intervals later in the season, after the plants began to show signs of recovery, the severity of symptoms on the original diseased parts of each plant was estimated, and the initiation and persistence of recovery, the amount of growth after recovery, and the degree of curly-top symptoms on recovered growth were recorded.

In 1942, similar studies were made on 3 groups of 35 Guasave-A plants each, inoculated on June 18, with curly-top virus strains 3, 9, and 58, respectively, which had also been included in the 1941 tests. Another group of 31 plants was inoculated by leaf hoppers from several stock colonies, each of which carried a different strain of curly-top virus. This population, composed of leaf hoppers carrying virus strains 3, 9, and 58, as well as several other strains known to infect tomatoes, was designated "composite strain mixture." A mixture of strains 3, 9, and 58 was used for inoculation of 5 additional plants: 5 leaf hoppers carrying strain 3, 5 carrying strain 5, and 5 carrying strain 58 were caged on each plant.

In both 1941 and 1942, there were some striking differences in the reactions of the Guasave-A plants inoculated with the different virus strains, and the differences observed between different strains were, in general, fairly consistent in the two years. In plants inoculated with a given single strain, however, the amount of injury before recovery, the time of initiation of recovery, and the degree of recovery were similar in the two years. In other words, the reaction of plants infected with a given strain in 1942 was similar to that of plants infected with the same strain in 1941.

As shown in table 2, the surviving plants in these tests were classified according to the amount of recovered growth, whether large, moderate, or small, and according to curly-top symptoms on recovered growth, whether mild, moderate, or severe. In interpreting these data, it must be kept in mind that there was considerable overlapping of the different classes. For instance, on some plants it was difficult to determine whether the amount of recovered growth was moderate or large and whether the symptoms were mild or moderate. The data show clearly that the strains of virus decidedly influenced the amount of recovery. With respect to the amount of recovered growth, plants infected with virus strain 3 were superior to all other groups. The severity of symptoms shown by the recovered plants in the strain-3 group was also, as a rule, noticeably less than that shown by the recovered plants of the strain-9 and strain-58 groups. Plants infected with strains 5, 9, 58, and 75, were, for the most part, in either the small- or the

TABLE 1.—*Development of curly-top symptoms and subsequent recovery, at Riverside, California, of Guasave-A tomato plants inoculated June 11, 1941, with different strains of the virus; 35 plants inoculated with each virus strain*

Date of observation and strain of curly-top virus	Number of plants affected	Original growth				Recovered growth	
		Symptoms	Stunting	Dieback	Fruitfulness	Stage of recovery	Symptoms
July 2, 1941							
Strain 3	34	Moderate	Medium	None	None	[a]	[a]
" 9	35	Moderate to severe	Medium to much	"	"		
" 58	35	Moderate	Medium	"	"		
" 75	32	Mild (as a rule)	Slight	"	"		
" 5	3	Extremely mild	None	"	"		
July 8, 1941							
Strain 3	35	Severe	Much	"	"		
" 9	35	Severe	Much	"	"		
" 58	35	Severe	Much	"	"		
" 75	34	Mild	Slight	"	"		
" 5	11	Very mild	None	"	"		
July 30, 1941							
Strain 3	35	Severe	Much	Little	Little	Early	
" 9	35	Very severe	Very much	Much	Little	Early	
" 58	35	Severe	Much	Much	Little	Very early	
" 75	35	Severe	Slight	Much	Little	Extremely early	
" 5	26	Mild	Very slight to medium	None	Medium		
Sept. 10, 1941							
Strain 3	35	Severe	[b]	Medium	[c]	Very advanced, very conspicuous	Mild
" 9	35	Very severe	Very much	Much	—	Advanced, conspicuous	Mild to moderate
" 58	35	Very severe	Much	Very much	—	Fairly advanced	Moderate
" 75	35	Very severe	Medium	Much	—	Early, variable	Moderate
" 5	28	Mild to moderate	Slight	Little	—	Very early, inconspicuous	Moderate to severe

[a] Blank spaces denote that no recovered growth, and hence no curly-top symptoms on recovered growth, were apparent; or that recovered growth was too slight for recording of symptoms.

[b] Stunting difficult to estimate because of large amount of recovered growth.

[c] Dashes indicate no data.

TABLE 2.—*Influence of virus strains on recovery of Guasave-A tomato plants from curly top*

Virus strain	Year	Plants inoculated	Plants infected[a]	Plants died from curly top		Distribution of surviving plants according to					
						Amount of recovered growth			Curly-top symptoms on recovered growth		
				Before recovery	After weak recovery	Large	Moderate	Small	Mild	Moderate	Severe
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
3	1941	35	35	0	0	22	11	2	13	22	0
	1942	35	35	0	0	20	7	1	20	7	1
9	1941	35	35	2	2	8	13	10	9	19	3
	1942	35	35	5	1	3	17	3	0	20	3
58	1941	35	35	2	5	3	13	12	7	19	2
	1942	35	35	1	3	0	2	21	—[b]	—	—
5	1941	35	27	0	0	7	6	14	0	16	11
75	1941	35	35	2	3	9	13	8	4	24	2
Mixture of 3, 9, and 58	1942	5	5	1	0	0	1	3	0	2	2
Composite mixture (many strains)	1942	31	31	3	2	1	3	21	—	—	—

[a] Of plants infected in the 1942 tests, a number died from Fusarium wilt and are therefore not included in the data in succeeding columns of this table.

[b] Dashes indicate no data. The slight amount of recovery and the severity of symptoms on recovered growth made it difficult to distinguish between new and old growth.

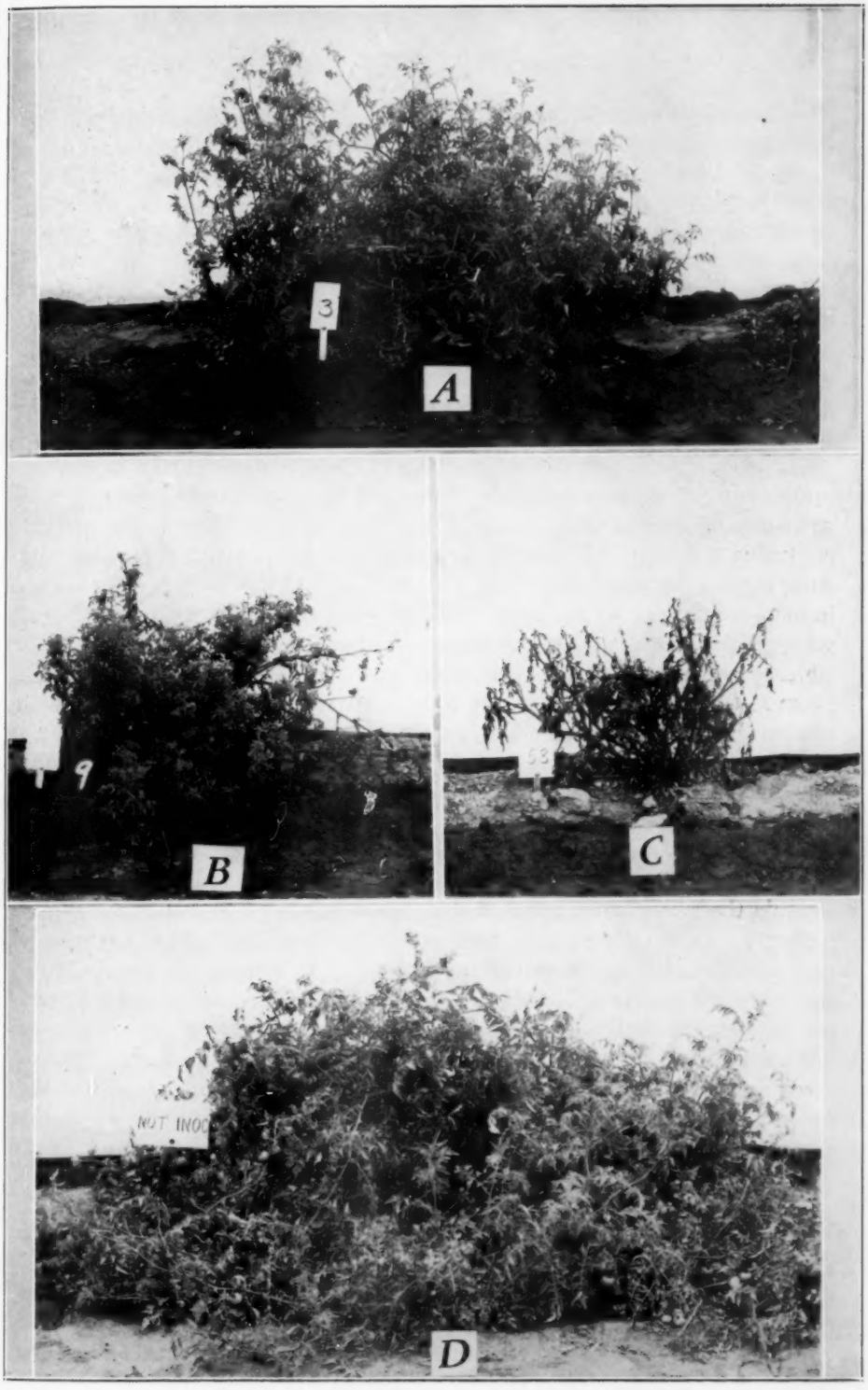

FIG. 1. The influence of different strains of the curly-top virus on recovery in Guasave-A tomato plants. A. Recovered growth of a plant infected with virus strain 3. B. Typical recovered plant infected with virus strain 9. C. Plant of group infected with virus strain 58, showing original severely diseased parts with slight recovery on the basal portions of the stems. D. Healthy, noninoculated plant. (Plants inoculated June 18, 1942; photographed September 17, 1942.)

moderate-growth classes; and comparatively few of the recovered plants of these groups were graded as having mild curly-top symptoms.

In the 1941 tests, recovery from strain 3 was more rapid, and the recovered growth was of a better quality, than in the case of plants infected with strain 9 or strain 58, even though all 3 strains caused severe injury during the early course of the disease. Recovery in plants infected with strain 9 was only slightly better than in plants infected with strain 58. The proportions of plants recovering were as follows: 100 per cent from strain 3, 88.6 per cent from strain 9, and 80 per cent from strain 58. Although virus strains 5 and 75 were not included in the 1942 tests, their behavior in comparison with that of the other strains, in 1941, gave further proof that, in this race of tomato, recovery varied with the strain of virus used.

In 1942, stunting of growth, due to curly top, was evident 18 days after inoculation. This was especially noticeable in plants inoculated with the composite mixture of virus strains and in those inoculated with the mixture of strains 3, 9, and 58. Plants inoculated with the individual strains were slightly less affected at that time. On August 31, 1942, 74 days after inoculation, recovery was by far the most advanced in the group of plants infected with virus strain 3, and was next best in the plants of the strain-9 group, which were, in general, in slightly better condition than those of the strain-58 group. On this date, the plants inoculated with the mixture of these 3 strains showed recovery about equaling that of the plants in the strain-58 group. Representative plants of the single-strain groups are shown in figure 1, A–C, in comparison with a healthy, noninoculated plant (Fig. 1, D). Earlier in the season, all the inoculated groups had shown very severe curly-top symptoms, consisting of almost complete defoliation and partial death of the stems. On September 16, 1942, the severity of the curly-top symptoms on the first-affected parts of the plants could still be estimated, except on the plants inoculated with virus strain 3, where those older parts were obscured by the large amount of new growth. In general, the condition of the recovered growth was more normal in plants that recovered earliest, and some plants, especially of the strain-3 group, produced a good crop of almost normal fruit on the recovered growth. Although all the different groups made more growth between the stage shown in figure 1 and the end of the season, the differences between the different groups remained in about the same order as that shown.

DISCUSSION AND CONCLUSIONS

In 1941, 5 strains, and in 1942 3 strains of the curly-top virus were used singly for inoculation of Guasave-A tomato plants. Since the percentage of inoculated plants that recovered was consistently high and probably not significantly different in the two years, it cannot be concluded that the variation in percentage recovery in 1939 and 1940 resulted solely from the use of different virus strains or strain mixtures. It is evident, however, that within a given year, Guasave-A tomato plants inoculated with different

strains of the curly-top virus varied widely in their recovery reactions. Even when the different virus strains caused severe initial symptoms resulting in marked injury to the inoculated plants, the time of the beginning of recovery and the amount and quality of recovered growth differed greatly. These differences were so great that it seems very probable that in some years environmental conditions may be the deciding factor in recovery of plants infected with some of the curly-top virus strains and yet may have very little effect on recovery from other strains. Recovery from virus strain 3 is initiated so rapidly and is of such high order as to suggest that, even under the conditions most favorable for development of curly top, plants affected by this strain would make a good recovery. On the other hand, in the case of certain other virus strains from which plants recover slowly and poorly, the same environment might cause a high mortality of plants, resulting in a low percentage of recovery.

In this study of recovery of Guasave-A tomato plants from curly top, the results support the earlier work of Wallace,[8] in which it was shown that acquired immunity from curly top in tomato may be of a high, intermediate, or low degree, dependent upon the virus strain carried by the immunized plants. Variations in recovery occurred between Guasave-A plants infected with strains of virus that cause equally severe symptoms on cultivated tomato varieties and similar initial injury on Guasave-A plants. It was of interest, however, that virus strain 5 was less virulent in its primary effect on Guasave-A plants than were the other virus strains used, but that recovery from strain 5 was inferior to that from strain 3, which caused severe injury in the early stages of infection.

While these investigations do not provide a conclusive explanation of the high interannual variability in the percentage of recovery of Guasave-A tomato plants, they suggest that the strain of virus, as well as the environmental conditions, determine the percentage of plants recovering. The data show further that, in investigations of recovery of tomato plants from curly top, it is essential that single virus strains, or known strain combinations, be used for inoculation of the plants under study.

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[8] See footnote 5.

A RAG-DOLL TECHNIQUE FOR THE INOCULATION OF WHEAT WITH BUNT (TILLETIA LEVIS)[1]

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INTRODUCTION

The difficulty experienced in obtaining high percentages of infection of greenhouse germinated and inoculated winter wheat with bunt (*Tilletia levis* Kühn) has discouraged detailed studies involving the host and parasite. The common method of dusting the chlamydospores on the seed and then planting in moist soil frequently failed to produce infection high enough to permit use of the data. The same difficulty was experienced when seed was germinated on blotting paper and transplanted to soil. The possibilities of the rag-doll method (1) for the germination of wheat became apparent during studies of the development of amylase in germinating wheat (4). Accordingly, tests were started in the fall of 1940 to determine the feasibility of using a rag-doll technique for germination of bunt spores and subsequent inoculation of sprouting wheat.

Holton and Heald (2) have reviewed the conditions that influence the germination of bunt spores and the infection of wheat plants. They report numerous investigations in which the highest average infection was obtained when inoculated seed was germinated at 10° C. This temperature was favorable for the germination of the chlamydospores (2), and, according to Kneen, Miller, and Sandstedt (4), it is favorable for the germination of wheat in rag dolls. They found that wheat could be left in the dolls at 10° C. for as long as 10 days before the sprouts became too long for transplanting; at the same time the temperature was sufficiently low to prevent contaminating molds from becoming a problem. Consequently, the first inoculation test reported in this paper was conducted at 10° C. and later investigations proved this temperature to be the most satisfactory.

MATERIALS AND METHODS

The rag doll used was modified from that prepared by Duddleston (1) by omitting the paper from around the cloth. Wrapping the cloth in paper occasionally interfered with the germination of the bunt spores. When the doll consisted of just a roll of cloth, germination was consistently high and infection of the susceptible wheat seedlings was adequate.

The dolls, after sterilization for 30 minutes at 15 lb. pressure in the autoclave, were laid out on a clean table and thoroughly soaked with tap water; then the excess water was removed by lightly scraping the cloth with a flat, wooden pot label. A few dry, glass microscope slides were placed on the cloth and the dry bunt chlamydospores[2] were dusted on them and

[1] From the Departments of Plant Pathology and Agricultural Chemistry, Nebraska Agricultural Experiment Station; published with the approval of the Director as Paper No. 336, Journal Series.

[2] The chlamydospores were a composite collection of *Tilletia levis* made by K. S. Quisenberry from the bunt nursery at Lincoln, Neb., in 1940.

the rag portions of the dolls. The dolls were then rolled moderately tight and placed in constant-temperature cabinets at 10° C. The extent to which germination had occurred was determined daily by removing a slide from each doll and observing it under the microscope. The chlamydospores usually began germinating on the fourth day and the first primary sporidia were observed on the seventh day. Since Sartoris (5) concluded that only primary sporidia were capable of infecting the host, the wheat was not added to the doll until primary sporidia were being produced. In this manner the sprouts could be exposed to the sporidia for the maximum time at this temperature. The wheat was prepared for placing in the dolls containing the germinating chlamydospores by carefully removing all broken and badly shriveled kernels and then soaking the seed in distilled water for 18 hours at room temperature. No other seed treatment was used. Two hundred seeds were placed, 1 to 0.5 in. apart, on the portion of the cloth dusted with chlamydospores; the doll was then rolled fairly tight, returned to the constant-temperature cabinet, and left for 10 to 14 days, depending upon the rapidity of sprout growth. It was found that seedlings with sprouts 30 mm. long were readily transplantable, but, with longer sprouts, the labor and time required to prevent high seedling mortality increased rapidly.

Moisture and temperature apparently were the most important factors to control in the rag-doll method. In 2 separate tests the dolls were permitted to become fairly dry for a few hours, and in both cases low infection resulted. The best results were obtained when the dolls were kept near saturaton. Drying was retarded by placing pans of water in the constant-temperature cabinet. The moisture content of the dolls may be readily adjusted by sprinkling.

INFLUENCE OF TEMPERATURE ON THE GERMINATION OF BUNT SPORES

The influence of various temperatures on the germination of bunt chlamydospores in rag dolls was studied by dusting the cloth portion of the dolls with chlamydospores then placing the dolls in constant temperature cabinets at 5, 10, 15, and 20 degrees C. Germination was most rapid at 10° and 15°, slightly slower at 20°, and still slower at 5° C. The production of primary sporidia was favored by the 10° C. temperature, first being observed after 7 days. They were produced in the same time at 15° C., but in much lower numbers.

In another test at 10, 14, and 18 degrees C., germination was first observed after 5 days at all temperatures, being 12 per cent, 19 per cent, and 1 per cent, respectively. Primary sporidia were observed at all temperatures on the 10th day, and after 12 days there was a much higher percentage of spores with sporidia at 10° C. than at the other two temperatures. It was difficult to obtain accurate counts of spores with sporidia because the sporidia would often be separated from the mycelium when unrolling the doll.

INOCULATION STUDIES

The results obtained in 3 separate studies demonstrated that the rag-doll method of inoculation is practical in tests where seedlings can be trans-

planted after inoculation. Ceres spring wheat was used in the tests reported; similar results, however, have been obtained with Cheyenne and Blackhull winter wheats.

In the first two inoculation trials (Tests 1 and 2, Table 1) the seed was placed in the dolls on the 7th day following introduction of the bunt spores, when primary sporidia were being produced, and left in the dolls for 12 days before planting in soil. In test 3 (Table 1) the seed was placed in the doll on the 10th day and left in the doll at 10° C. for 12 days, when half of

TABLE 1.—*The degree of bunt infection obtained with Ceres spring wheat by using the rag-doll procedure for seedling inoculation*

Inoculation method	Number of plants headed[a]	Number of seedlings	Number of plants smutted		Percentage of plants smutted	
			Head count	Seedlings with chlorotic spots[b]	Head count	Seedlings with chlorotic spots
Test 1						
Rag doll[c]	127	127	115	109	95.5	85.8
Chlamydospores on seed[d]	130	130	42	47	32.3	36.0
Doll control[e]	88	89	0	2	0.0	2.2
Control—seed planted in soil	97	97	0	1	0.0	1.0
Test 2						
Rag doll	137	137	124	121	90.5	88.3
Doll—control	46	46	0	0	0.0	0.0
Test 3						
Rag doll—seedlings planted in sand	395	428	230	284	58.2	66.4
Rag doll—seedlings planted in soil	61	63	36	38	59.0	60.3
Control—seedlings planted in sand	36	36	0	0	0.0	0.0

[a] Due to some Helminthosporium seedling blight in test 3, a few plants died before heading. These were included in the seedling count.

[b] Chlorotic spots resulting from bunt infection as described by Johnston and Lefebvre (3).

[c] Bunt spores placed in rag doll and held at 10° C. until primary sporidia produced, then soaked Ceres spring wheat placed in doll until sprout was 30 mm. long. Sprouted seed planted in soil unless otherwise stated.

[d] Dry chlamydospores were dusted on the seed and the seed planted in flats of soil with a moisture equivalent of 12.76. The moisture was maintained at about one and one-fourth moisture equivalent and the flats kept in the greenhouse at 10° C.

[e] Seed germinated in doll, not inoculated.

the seedlings were planted in sand cultures and half in soil. Noninoculated controls, in which the seed was germinated in sterilized rag dolls, then planted in soil or sand, were used in each test.

The results of the inoculation tests presented in table 1 are recorded on the basis of the number of plants with smutted heads and the number of seedlings with the chlorotic spots typical of bunted plants. These chlorotic spots have been described by Tillet (6) and more recently by Johnston and Lefebvre (3).

There was a marked contrast between the amounts of infection obtained with the rag-doll inoculation and the procedure involving the dusting of chlamydospores on the seed and planting directly into the soil (Test 1). The low infection obtained by the latter method is typical of that obtained in previous years. The rag-doll method also gave a high percentage of infection in test 2, where the plants were grown in temperature cases at an air and soil temperature of 21° C. after the inoculation at 10° C.

FIG. 1. Stunting of wheat plants caused by bunt infection of seedlings and subsequent growth in sand culture. 7C—control. 7—inoculated.

The chlamydospores germinated slowly in test 3 and the infection was also reduced from the previous tests. The reason for the reduced infection is not known. However, the degree of infection was adequate for the experimental purpose. It made very little difference whether the inoculated seedlings were planted in sand or soil, except that the chlorotic leaf spots were evident earlier and were much more pronounced in the sand cultures. Under certain nutritional treatments in sand culture, stunting of the inoculated plants occurred. Figure 1 shows a comparison of the control and the inoculated plants grown under one of these treatments.

VALUE OF THE LEAF-MOTTLING CHARACTER

The chlorotic spots on the leaves of bunted seedlings were found to be valuable in making preliminary determinations of the amount of infection obtained. As can be seen from the table, there was not complete agreement between the number of seedlings with chlorotic spots and the appearance of smutted heads on the plants. A few of the seedlings showing the chlorotic spots that were typical of those on the leaves of bunted plants died from *Helminthosporium* infection, particularly in sand culture (Test 3). There were also a few cases in which it was difficult to distinguish between the spots and insect injuries. Then, too, it is entirely possible that the bunt infection may have encouraged the death of some of the seedlings before heads were produced. However, the data in table 1 indicate that with the variety Ceres it is possible to use the presence of chlorotic spots as an indication of the degree of infection and as a criterion on which to base continuance or early termination of an individual study. Similar data (unpublished) indicate the same to be true for two winter wheat varieties, Cheyenne and Blackhull.

SUMMARY

The rag-doll method of inoculation was found to be particularly well adapted to studies where wheat seed is to be germinated and inoculated with bunt under uniform conditions. A large number of seedlings can be inoculated with a minimum of labor and the resulting seedlings are in an excellent condition for transplanting. High degrees of infection were obtained by pregermination of the bunt spores in rag dolls followed by the addition of soaked wheat seed to the same dolls upon the appearance of primary sporidia. Seven to 10 days at 10° C. were required for adequate germination of the bunt spores followed by a 10- to 14-day period at the same temperature to permit the germinating wheat to produce sprouts of some 30 mm. length. It was found that such seedlings could be transplanted into either sand or soil with facility and in the case of the controls, with subsequent normal development.

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PHOMA TERRESTRIS ON GRAMINEAE IN THE NORTHERN GREAT PLAINS[1]

RODERICK SPRAGUE

(Accepted for publication August 4, 1943)

Kreutzer[2] demonstrated in 1941 that *Phoma terrestris* Hansen, which causes pink root of onion, could, under artificial conditions, attack certain cereals. In 1942, Fischer *et al.*[3] reported *Agropyron smithii* Rydb. as a natural host for *P. terrestris*, as determined by isolations made by the writer, and in 1943, Johann[4] listed corn as a host for this fungus. On the basis of studies during the past 4 years, it appears that *P. terrestris* should be recognized as a widespread but minor weak parasite and saprophyte on the underground parts of cereals, many grasses, and certain other hosts in North Dakota and adjoining States. During the 3-year period, 1940–1942, it was isolated 366 times, as well as a number of times in 1943.

During 1940 to 1942, the fungus represented only 2.3 per cent of 16,086 pure cultures of all species of fungi obtained by the writer from the roots of field-grown plants. However, while this fungus is seldom dominant in root material, it appears on a wide range of hosts. Isolations have been made from the following 55 gramineous hosts in this region: *Agropyron cristatum* (L.) Gaertn., *A. michnoi* Roshev., *A. repens* (L.) Beauv., *A. semicostatum* (Steud.) Nees, *A. sibiricum* (Willd.) Beauv., *A. smithii*, *A. trachycaulum* (Link) Malte, *Andropogon furcatus* Muhl., *A. hallii* Hack., *Arthraxon hispidus* var. *cryptatherus* (Hack.) Honda, *Avena sativa* L., *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis* (H.B.K.) Lag., *Bromus arvensis* L., *B. carinatus* Hook. and Arn., *B. erectus* Huds., *B. inermis* Leyss, *Dactylis glomerata* L., *Distichlis stricta* (Torr.) Rydb., *Echinochloa crusgalli* (L.) Beauv., *Elymus canadensis* L., *E. dahuricus* Turcz., *E. excelsus* Turcz., *E. interruptus* Buckl., *E. junceus* Fisch., *E. sibiricus* L., *Eragrostis pilosa* (L.) Beauv., *Festuca octoflora* Walt., *F. rubra* L., *Hordeum brevisubulatum* (Trin.) Link, *H. distichon* L., *H. vulgare* L., *Koeleria cristata* (L.) Pers., *Muhlenbergia richardsonis* (Trin.) Rydb., *Oryzopsis hymenoides* (Roem. and Schult.) Ricker, *Panicum capillare* L., *P. miliaceum* L., *P. virgatum* L., *Phleum pratense* L., *Poa pratensis* L., *P. secunda* Presl, *Roegneria pendulina*

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, and Dry Land Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; and Division of Nurseries, Soil Conservation Service, U. S. Dept. of Agriculture; and the North Dakota Agricultural Experiment Station.

[2] Kreutzer, W. A. Host-parasite relationships in pink root of *Allium cepa*. II. The action of *Phoma terrestris* on *Allium cepa* and other hosts. Phytopath. 31: 907–915. 1941.

[3] Fischer, George W., R. Sprague, H. W. Johnson, and J. R. Hardison. Host and pathogen indices to the diseases observed on grasses in certain western States during 1941. U.S.D.A. Pl. Dis. Rptr. Suppl. 137. Aug. 15, 1942. (Mimeographed.)

[4] Johann, Helen. *Phoma terrestris* in the roots of mature maize plants. Phytopath. 33: 526–528. 1943.

Nevski,[5] *Schedonnardus paniculatus* (Nutt.) Trel., *Setaria italica* (L.) Beauv., *S. lutescens* (Weigel) F. T. Hubb., *S. viridis* (L.) Beauv., *Stipa comata* Trin. and Rupr., *S. viridula* Trin., *Sorghum vulgare* Pers., *S. vulgare* var. *sudanense* (Piper) Hitch., *Triticum aestivum* L., *T. durum* Desf., *T. dicoccum* Schrank, *T. timopheevi* Zhuk., and *Zea mays* L. We have also isolated *P. terrestris* from *Allium cernuum* Roth, *Cucurbita pepo* L., *Linum usitatissimum* L., and *Lepidium campestre* (L.) R. Br.

Although the roots of wheat growing in dry sandy soil in mid-late season afford the most common host for *Phoma terrestris*, it also is associated with a seed and seedling disease of corn, sorghum, and certain grasses earlier in the year. This latter condition was particularly common during June, 1943, at Mandan, N. Dak., when the precipitation for that month totalled 7.67 inches.

Trials in the greenhouse with soil-bran, pure-culture inoculum introduced at seeding time into electrically sterilized loam soil in redwood flats showed that *Phoma terrestris* is, at best, a very weak parasite. While strong pigmentation associated with mild lesions developed on the outer tissues of some hosts, no deep penetration of healthy tissue occurred. The first two triplicated inoculation trials were completely negative, except for superficial coloring of some of the roots and sub-crown internodes of *Agropyron cristatum, Elymus junceus, Poa pratensis* L., Rainbow oats, Wisc. 38 barley, and Pilot and Kubanka wheats. These were seeded in the greenhouse on Nov. 9, 1940, and examined on Jan. 20, 1941. The temperature in the greenhouse during this period ranged from 56° to 66° F. One of the two series was inoculated with *Phoma terrestris*, which had been originally isolated from Gopher oats, Dickinson, N. Dak., in July, 1941 (culture 212 A–1), and the other from Spartan barley (culture 214 A–1) from the same place. Both of these isolates had been obtained from maturing plants growing in dry soil. A third inoculation was made in the greenhouse in Nov., 1941, and grown at a slightly lower temperature (52° to 60° F.). The inoculated seedlings were examined Dec. 9, 1941. They showed the following reduction in yield of air-dried plants when compared with noninoculated healthy checks: *Agropyron cristatum*, trace; *A. trachycaulum*, 0.3 per cent; Rainbow oats, trace; *Bouteloua gracilis*, 10.5 per cent; *Bromus inermis*, trace; *Elymus junceus*, trace; Wisc. 38 barley, trace; Turghai proso, 1.3 per cent; Sudan grass, 4.4 per cent; Pilot wheat, none; and Golden Tom Thumb pop corn, trace. The culture in this third series was obtained from a seedling of *Agropyron smithii* at Mandan, N. Dak., May 15, 1941, and it was sufficiently pathogenic to merit listing in the 1942 check list[6] of the parasitic fungi on Gramineae. However, it should be emphasized that this fungus caused only 10.5 per cent loss in the small seeded grass, *Bouteloua gracilis*. Under the same artificial conditions, *Pythium arrhenomanes* Drechs. and *Pythium debaryanum* Hesse, usually caused complete preemergence death

[5] According to J. R. Swallen, *Roegneria pendulina* Nevski should be transferred to *Agropyron*, but the transfer has not yet been made.

[6] See footnote 3.

of this grass. *Phoma terrestris* is, therefore, considered to be a weak parasite on grasses and probably pathogenic only when the hosts are growing slowly in cool (about 50° F.) wet soil. The pink root condition in maturing cereals represents a saprophytic development on small roots killed by either *Pythium arrhenomanes,* other fungi, or by drought. It is evidently much less common than the pink root on mature cereals and grasses, induced by *Fusarium oxysporum* (Schlecht.) em. Snyder and Hans.

Phoma terrestris growing on potato-dextrose agar forms compact, slow growing, somewhat mounded velvety, gray colonies with rose-colored, plum or vinaceous tints, particularly in the substratum. The colonies are often sterile, particularly when incubated in total darkness in refrigerators. They vary considerably in amount of coloration and several distinct races are distinguishable. In 1940, J. E. Machacek told the writer that these cultures, which were sterile at that time, were probably a species of *Phoma.* In the fall of 1940, one culture isolated from *Elymus sibiricus* (199 A–2) produced elliptical, hyaline pycnospores, 3–4.8 × 1–1.5 μ. Later, typical cultures, obtained from *Agropyron cristatum, A. sibiricum,* and *Triticum aestivum,* and grown either on melilotus culms or on corn meal in flasks, produced spores similar to those of culture 199 A–2 when held under common storage conditions. The sterile, gray and rosy-tinted cultures are, however, so characteristic that little difficulty is encountered in distinguishing them from other fungi isolated from diseased roots of Gramineae.

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OBSERVATIONS ON A GALL OF SUGAR MAPLE

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(Accepted for publication June 1, 1943)

INTRODUCTION

Burls or galls of varying sizes and shapes are common on hardwoods. Certain of these burls are noninfectious and apparently result from injuries, while others are known to be caused by bacteria or fungi.[3] Globose or fusiform galls, often bearing one or more cankers, are fairly common on the trunks of sugar maple (*Acer saccharum* Marsh.) growing on the Kane Experimental Forest, Pennsylvania, and on the adjacent Allegheny National Forest. Similar galls also were observed in New York and New England. Lorenz and Christensen[4] have referred to the same type of sugar-maple gall as "globose canker." The cause of these galls is unknown, and the purpose of this paper is to describe them pending further investigations.

DESCRIPTION OF THE SUGAR MAPLE GALL

Sugar-maple galls were observed on trees from 2 to 18 inches d.b.h. with most affected stems from 4 to 8 inches in diameter. Practically all the galls developed on the main trunk, usually not over 6 to 8 feet from the ground. The number of galls on an affected tree ranged from 1 to as many as 4. If a sprout clump was affected it was not uncommon to find most of the stems in the clump bearing one or more burls. Trees with galls were not uniformly distributed throughout the forest, but usually occurred in groups of 2 or 3 affected trees in an area small enough to be readily noted from a fixed point of observation.

The gall starts as a localized outgrowth involving the whole diameter of the stem at the affected spot or only a part of the diameter, and results in a pronounced swelling (Fig. 1, A and D). In the early stages of gall formation the bark over the hypertrophied part is often smooth, or with only shallow ridges, and generally appears to be uninjured. On the larger galls the bark is usually cracked considerably on the part showing the greatest distortion. Dead streaks often develop on one or more sides of the swollen area, making the gall appear as a globose canker. The cankerous aspect of the galls usually increases with age, or at least with size, and the larger galls often have 2 or 3 open dead areas. Some of these dead areas finally heal

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[3] Brown, Nellie A. Tumors on elm and maple trees. Phytopath. 31: 541–548. 1941.

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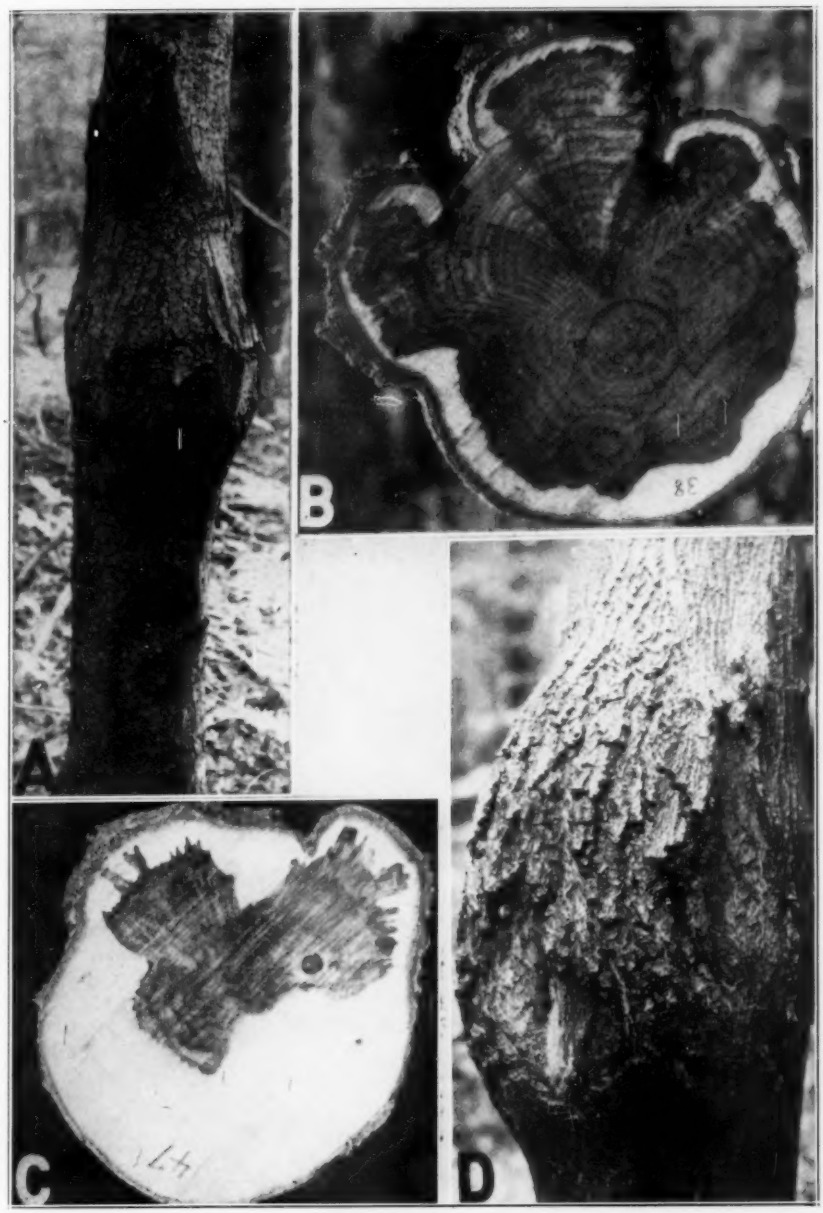

FIG. 1. Galls of sugar maple (*Acer saccharum* Marsh.). A. A typical swelling on young stem. B. Cross section of a gall showing extensive dark-colored internal area. C. Cross section of one-sided gall showing inner discoloration more extensive in swollen areas. D. A gall with two dead areas.

over, producing elongated ridges. The surface of such a gall seldom remains unbroken long, since further activity by the agent responsible for the swelling causes other dead streaks, cracks, or cankers to develop. In time, persistent dying of the cambium may kill the tree by girdling.

Cross sections through galls revealed that the wood associated with the hypertrophied part was dark, stained with greenish-black streaks or blackish streaks in a reddish-brown background in contrast to the normal white wood of maple. Sections through galls developing as one-sided swellings on the trunk illustrates this clearly, since the wood on the unaffected side remained white, while that at the center of the swelling was stained, the pattern of the stained part corresponding in general to the shape of the swelling (Fig. 1, C). Where the gall involved the whole diameter of the stem the discoloration extended throughout the entire cross-sectional area with the exception

FIG. 2. Part of a longitudinal section of a small gall, showing restriction of zones of discolored wood to area of swelling.

of a narrow zone of sapwood (Fig. 1, B). On galls that had not developed extensive cankering it was observed that on the cross sections fingers of greenish stain extended toward the cambium from the mass of discolored wood at the center (Fig. 1, C). Wherever these fingers or outgrowths of stained wood touched or closely approached the cambium, the latter was killed, resulting in cracks or dead streaks on the surface of the gall (Fig. 1, B and D). The greenish or blackish discolorations extended only a short distance up and down the stem and were confined between the upper and lower limits of the swollen area (Fig. 2). This dark-stained wood remains very hard and heavy and is remarkably similar to what has been called mineral stain.

CAUSE

Numerous attempts to isolate a causal organism from the interior of this type of gall have given inconsistent results. No organism was obtained from the outer margin of the discoloration, that is, from the outer one-half to one-fourth inch of dark wood that was covered by a thin layer of living sapwood. Several organisms were usually obtained from the central area of discoloration. Most common among these were a slow-growing bacterium, similar to one isolated from redheart in birch,[5] and *Torula ligniperda* (Willk.) Sacc. Occasionally the conidial stage of *Coniochaeta* sp., a faster spreading bacterium, and *Coryne sarcoides* (Jacq. ex Fr.) Tul. were isolated from the same central or pith region of the discolored area. These organisms were like those consistently isolated from normal central discolorations in maples and birches.

The phomopsislike fungus reported by Brown[6] was never isolated from the galls. *Nectria* sp., although occasionally found fruiting on dead areas, was never isolated from the interior tissue.

There is usually very little evidence of a point of origin at the center of these galls, although it is supposed that there is some slight defect such as a small branch stub, insect injury, ring shake, or other dead areas as a point of origin.

If any of the organisms isolated is responsible for the death of the sapwood its effect must be indirect, because none of them was isolated from the advancing margin of diseased tissue. Another interesting point is that the pathological activity continues in narrow strips, on both sides of which dead areas have developed in the cambium (Fig. 1, B). The high moisture content of the stained wood might, through frost or extreme cold, have a detrimental effect on the living surrounding cells, but this has not been proved. Regardless of the actual cause, the gall appears to be caused by some infectious agent that gets into the stem when the trees are small and slowly works outward through the living sapwood and encroaches slowly to within one to several millimeters of the cambium with a resultant stimulation to growth at that point.

IMPORTANCE OF THE SUGAR MAPLE GALL

No estimate was made as to the percentage of affected trees on the Kane Experimental Forest, but the galls were "common," meaning that they occurred throughout the forest in numbers sufficiently great to be readily noticeable. Many of the trees with galls were in the intermediate or suppressed groups, and such trees usually are eliminated in the long run by natural thinnings. For practical purposes a tree bearing this type of gall has little promise of producing timber and may be removed if cuttings for chemical or fuel wood are being made. Evidently the agency inducing gall formation is not highly virulent, for the increase in their number in a stand takes place at a slow rate.

[5] Campbell, W. A., and R. W. Davidson. Redheart of paper birch. Jour. For. 39: 63–65. 1941.

[6] See footnote 3.

SYNERGISM AS A TOOL IN THE CONSERVATION OF FUNGICIDES[1]

ALBERT E. DIMOND AND JAMES G. HORSFALL

(Accepted for publication July 15, 1943)

Our country relies heavily on copper in the control of plant diseases. In the past year, 100,000,000 lb., computed as copper sulphate, were used in agriculture. Copper is much in demand in time of war. Plant pathologists are constantly on the alert to develop techniques for reducing the quantity used in combating plant diseases without sacrifice in level of control. It is the purpose of this note to point out the possibilities of using synergistic systems of fungicides to conserve copper.

For the past year the writers have been engaged in an examination of the extent to which savings in fungicides can be effected through synergism. A typical system showing synergism consists of carbon disulphide and dimethyl amine as members,[2] and results of a study of this system are presented in Table 1. The magnitude of savings possible through use of systems of poisons displaying synergism will be apparent.

TABLE 1.—*Relative dosage necessary to inhibit 50 per cent of a population of spores of Sclerotinia fructicola using a synergistic system*

Composition of poison		Relative quantity to give 50 per cent inhibition
Per cent Carbon disulphide	Per cent Dimethyl amine	
100.0	0.0	660.00
94.3	5.7	2.52
85.9	14.9	1.78
72.9	27.1	1.06
63.2	36.8	1.00
50.0	50.0	1.01
30.8	69.2	1.34
0.0	100.0	61.50

An illustration of synergism resulting from mixtures of two materials, only one of which shows toxicity to the test organism, is the system consisting of cuprous oxide and elemental sulphur. The dosages of these materials necessary to cause a 50 per cent inhibition of *Macrosporium sarcinaeforme* are shown in table 2. A distinction should be clearly made between synergistic and additive action of fungicides. That the system under discussion is synergistic rather than additive can be demonstrated from the fact that sulphur alone is not inhibitory to spores of *Macrosporium*, whereas combinations of copper and sulphur are more toxic than is copper alone. From the

[1] Contributions from the Department of Botany, University of Nebraska, N. S. No. 140, and of the Connecticut Agricultural Experiment Station. Published with the approval of the Director.

[2] The mechanism underlying synergism for this system is chemical, as will be shown in a subsequent paper.

point of view of the savings of total dosage of fungicide (copper plus sulphur), these data indicate that the phenomenon may be put to practical use, for, with this synergistic system, only half as much total fungicide is required for a given level of parasite control as when pure toxicants are employed. Furthermore, the savings in copper, the critical material in this system, is 10-fold.

Table 2 illustrates some interesting properties of synergistic systems. When both fungicides used in the system are plentiful, it will be most practical to utilize members at the composition that give maximum toxicity, *i.e.*, at the composition requiring a minimum total quantity of fungicide for a given level of parasite control. When one is plentiful and the other is not (and in this case cost is proportional to scarcity), it will be more economical and will conserve the materials needed in other ways to use the composition

TABLE 2.—*Savings in a copper fungicide made possible through using it as a member of a synergistic system against spores of Macrosporium sarcinaeforme*

Composition of poison		Total quantity of fungicide for 50 per cent spore inhibition (γ per cm.^{2})	Dosage of Cu_2O for 50 per cent inhibition (γ per cm.^{2})
Per cent cuprous oxide	Per cent sulphur		
100	0	5.00	5.00
97	3	3.41	3.31
90	10	2.50	2.25
70	30	2.12	1.48
50	50	2.12	1.06
30	70	2.27	0.68
10	90	4.93	0.49
3	97	75.80	2.27
0	100	Infinite	.....

giving a maximum saving of the critical material. The peak of toxicity as composition is varied will not necessarily be the composition most desirable from the standpoint of cost or of conservation of copper. In table 2, it may be seen that a composition consisting of 70 per cent copper and 30 per cent sulphur will be cheapest to apply and conserve the most copper.

It is evident that the savings of fungicide that can be effected through use of synergistic systems can be appreciable and may prove practical under field conditions.

The above data were obtained in the laboratory. Tests of synergism between cuprous oxide and sulphur as spray materials under field conditions in 1942 were made for control of potato tip-burn, a disease of uncertain etiology. Each mixture was applied in 3 different dosages, and each dosage was replicated in 5 randomized blocks (Table 3).

In Table 3 it is apparent that a mixture containing 75 to 90 per cent of cuprous oxide and 10 to 25 per cent sulphur gave the maximum protective value against tip-burn as compared with 70 per cent cuprous oxide and 30 per cent sulphur in the laboratory.

When considered in the light of the war-short material copper, the mix-

ture that used the least copper was that containing only 25 per cent cuprous oxide. Stated otherwise it means that if 75 per cent of the cuprous oxide was replaced with sulphur, the action of the cuprous oxide was synergistically improved some 14-fold. This is of the order of the 10-fold improvement in fungicidal action noted in the laboratory. Sulphur and copper have been used periodically together in sprays since Millardet first discovered Bordeaux mixture. In no case have the results distinguished additive from synergistic action. In the present case, sulphur alone was almost valueless in tip-burn control, so that synergism is clearly indicated.

No synergism between cuprous oxide and sulphur was observed in 1942 field tests on celery for control of *Septoria apii*.

Since there is at present no way of determining on an *a priori* basis what systems of poisons will be synergistic or the magnitude of synergism that

TABLE 3.—*Savings in a copper fungicide made possible through using it as a member of a synergistic system against potato tip-burn in the field*

Composition of poison		Dosage for 50 per cent tip-burn control (pounds per acre)	
Cuprous oxide (per cent)	Sulphur (per cent)	Mixture	Cuprous oxide only
0	100	224.00[a]	.....
10	90	19.40[a]	1.94
25	75	4.57	1.16
50	50	3.15	1.56
75	25	2.94	2.21
90	10	2.75	2.48
100	0	190.00	16.40

[a] Obtained by extrapolation.

will be shown by given poisons acting jointly, one can merely examine systems in a wholesale manner with the object of finding correlations and underlying mechanisms for the phenomenon. A considerable number of 2-member systems have been examined, and the following list indicates some possibilities: cuprous oxide with metallic oxides (ZnO, PbO, etc.), metallic oxides with sulphur, mercaptobenzothiazole with sulphur, and diphenyl amine with sulphur.

Yet another potentiality in the use of synergistic systems lies in the use of organic fungicides. An obstacle to the use of these materials as foliage sprays is their high cost and the fact that their production is limited by facilities of existing plants. Using organic fungicides as members of synergistic systems offers the possibility of reducing the cost of spray treatment and likewise of extending existing supplies.

Finally, it may be stated that systems of poisons synergistic for a single organism are not necessarily synergistic for all organisms. The system cuprous oxide-sulphur has been examined using two fungi: *Macrosporium sarcinaeforme*, susceptible only to copper, and *Sclerotinia fructicola*, susceptible to both toxicants. Preliminary results indicate that the increase in

toxicity against *Sclerotinia* is by no means so pronounced as it is against *Macrosporium*. Likewise, in the field, the cuprous oxide-sulphur system was synergistic in the control of potato tip-burn but not in the control of *Septoria apii* on celery.

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CRANBERRY FALSE BLOSSOM IN RELATION TO FLOODING WATER

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(Accepted for publication August 18, 1943)

The few Wisconsin cranberry marshes on which no case of the false-blossom disease has been observed on susceptible varieties during the past 7 years are chiefly those with alkaline flooding water. In at least 2 cases we have definite records of the introduction of infected vines of susceptible varieties on portions of such marshes where the disease is certainly less abundant than it was formerly.

This has occurred during a period when in spite of serious and apparently largely successful efforts at the control of the only known insect carrier, false blossom has in general increased on Wisconsin marshes. This opinion, namely, that, in spite of our best efforts at control, false blossom has been on the increase in Wisconsin during the past 7 years, is shared by

TABLE 1.—*Marshes with alkaline flooding water on which no false blossom has been found 1937–1943*

Location	Varieties	pH of flooding water
Hertel	Searls	7.6
Rice Lake	Bennett & Searls	7.4 – 8.8
Westfield	Searls	7.4 – 8.1
Berlin	Howes & Natives	7.5 – 8.2
Waupaca	Searls & Natives	7.8 – 8.5
Spooner	Searls	7.6
Spring Brook	Howes & Searls	7.5

other observers. It was, for example, voiced by Vernon Goldsworthy, who, as manager of the Wisconsin Cranberry Sales Company, has been a constant and keen observer of Wisconsin marshes for the last 10 years, in his Circular Number 40, dated June 23, 1941.

As is well known, false blossom is present on the vast majority of Wisconsin cranberry marshes. On the other hand, almost the only cases where marshes with very alkaline water are known to contain plants affected with false blossom are new marshes where false blossom plants have been recently set. The foregoing observations so strongly suggest a possible causal relation that they seem worthy of brief record, even though they are unsupported by experimental evidence. Moreover, if a causal relation could be demonstrated it would have no obvious commercial importance, for too many difficulties seem to be associated with the use of such water. The flooding water of the great majority of Wisconsin marshes is between pH 6.0 and pH 7.0.

Negative evidence, particularly negative field evidence, is usually not taken very seriously. The symptoms of cranberry false blossom are, how-

ever, conspicuous and the disease has been a major interest of mine for at least 20 years. It seems reasonably certain, then, that it is not abundant on these marshes, which have been repeatedly visited and studied during the period under discussion.

It is possible that the disease may never have been introduced on the marshes at Hertel, Westfield, and Waupaca. The only Searls vines planted on them are known to have come from what is still one of the cleanest marshes in the State. On the other hand, it is highly probable that diseased vines have been planted on each of the others and evidence that the disease was introduced on the Berlin Marsh in considerable amounts in vines of the susceptible Howes variety seems to be beyond question.

Under date of October 17, 1930, Mr. L. M. Rogers, then Cranberry Specialist for the Wisconsin State Department of Agriculture, made the entry in his notes with reference to this marsh, "False blossom well scattered through the Howes. I did not see it on the Natives." The Howes vines

TABLE 2.—*Marshes with alkaline flooding water where false blossom has been recently introduced*

Location	Variety	pH of flooding water
Hayward	Searls	7.9
Court O'Relles	Searls	7.2 – 7.9

here mentioned were planted about 1925, and came from New Jersey. They were almost certainly infected when planted. Later, under date of June 9, 1931, Mr. Rogers again notes the apparent absence of false blossom on the Natives and its presence in considerable abundance on the Howes. On August 30, 1933, May 17, 1935, and July 26, 1935, he mentions specifically looking for symptoms of the disease and failing to find them in the vicinity where they were found in 1930 and 1931. In the last record he adds "I think most of the false blossom that came in the Howes has died out." To this I can only add that I have been unable to find the disease here during 1937–1943.

There are other marshes than those listed that indicate alkaline flooding water does not favor the spread of cranberry false blossom. On the marsh near Turtle Lake (flooding water 7.4 – 8.0) there were planted in 1932, 5 acres of Howes vines known to have come from a marsh where there was and is a scattering infection of false blossom. Moreover, on August 31, 1935, L. M. Rogers entered the record "False blossom present in many places." Careful search on these same sections under favorable conditions in 1941, 42, and 43 revealed only a few uprights showing symptoms of false blossom. On a marsh near Nekoosa (flooding water 7.3), Mr. Rogers spent nearly an entire day in 1935 roguing false-blossom plants from a one-fourth acre section of somewhat mixed McFarlin vines planted in 1934. Since 1937, I have been able to find on this section only a single diseased upright. Even if it be assumed that Mr. Rogers removed every infected shoot but one in 1935, it is obvious that the disease has not spread.

The relation indicated by the foregoing observations suggests that these alkaline marshes, often not very productive of fruit, might be used for growing vines for sale as planting stock but for the psychological difficulties of selling vines from areas known to be relatively unproductive.

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TILLETIA TUMEFACIENS, A REMARKABLE GALL-FORMING SMUT FROM INDIA

B. B. MUNDKUR

(Accepted for publication July 2, 1943)

Tilletia tumefaciens Sydow was founded by Sydow (1) to accommodate a smut occurring in the shoots and axillary buds of *Panicum antidotale* Retz. It was collected at Lyallpur on Sept. 30, 1908, and was sent by D. Milne to Sir Edwin J. Butler who diagnosed it as an undescribed species of *Tilletia*. The specimen in the Herb. Crypt. Ind. Orient. is a small portion of the

FIG. 1. Healthy *Panicum antidotale*, showing rhizome-like base.

scanty-type specimen, the major portion of which is in the Herb. Sydow at Stockholm. A description of the fungus recently was published by Mundkur (4).

Abundant material of this unique gall-forming *Tilletia* has recently been collected at Rohtak, a small township about 30 miles due west of New Delhi, which has enabled a more careful study of it to be made.

Panicum antidotale is a tall perennial grass with a stout rootstock; it is often found growing among the shrubs in hedges or as isolated bushes. Its

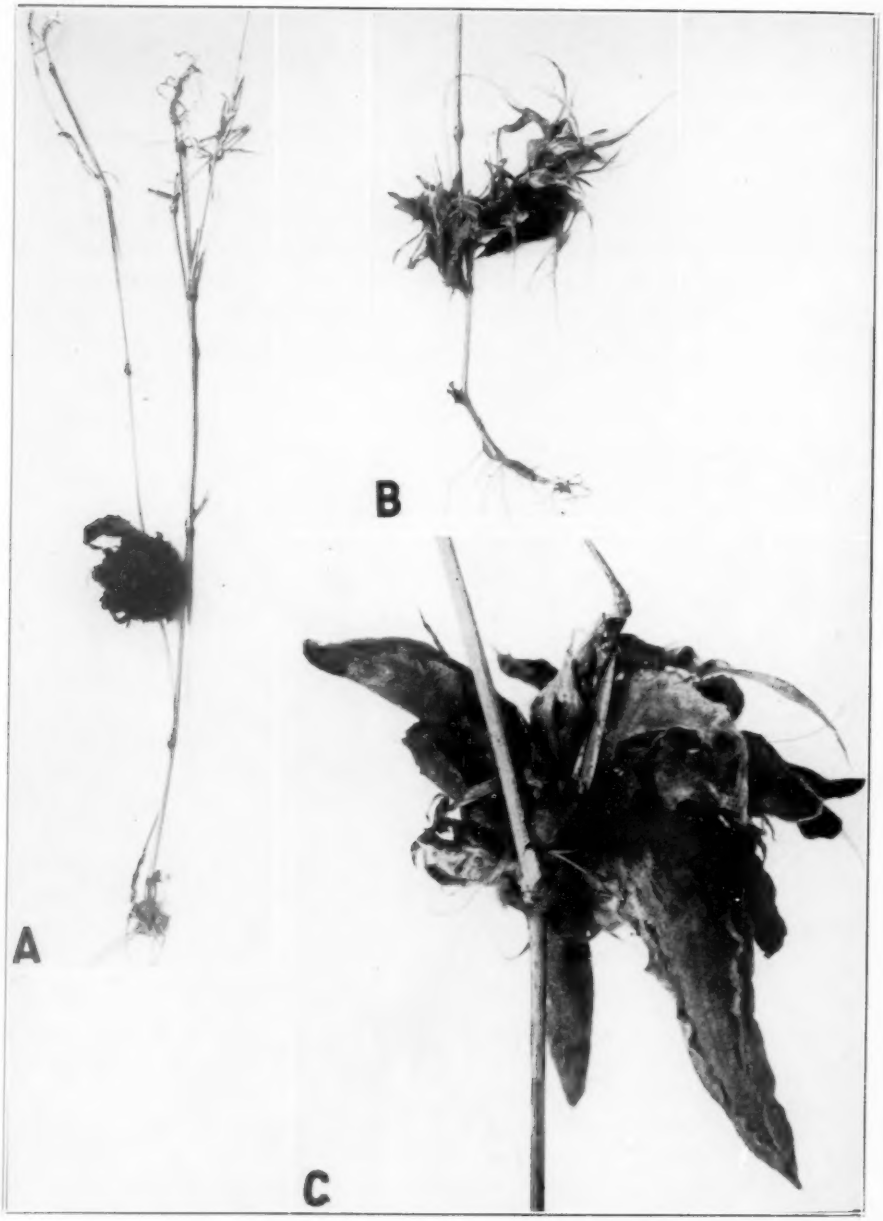

FIG. 2. A. Axillary buds turned into galls at the base of the plant. B. Witches broom effect; all leaves are not attacked. C. Buds transformed into large, finger-like tumors.

culms attain a height of 1½ m., are solid, terete and woody, and its leaves are rigid, linear, up to 60 cm. long and 2 cm. wide, with a smooth surface on both sides. Intra-vaginal branches arise from the leaf axils, especially at the base

of the plant (Fig. 1). The growing point of the young undeveloped branches is enclosed by smooth bud scales, about 2 cm. long. The grass is said to have a medicinal value in throat affections and for fumigating wounds; it also is stated to be an antidote for hydrophobia.

In a smutted plant (Fig. 2), the young axillary buds at the base are attacked, the main shoot, bearing undamaged inflorescence, being healthy. The upper axillary buds, as a rule, are not affected, though a gall also was found high up on the grass. The attacked bud, the bud scales and the prophyllum, as a result of hypertrophy and hyperplasia, become considerably swollen, forming a globose, black mass, having the appearance of a gall, though the structure actually consists of several galls held together. In advanced cases (Fig. 2, C), the central bud enlarges, being 9 to 10 cm. long and 2 to 3 cm. broad, forming a finger-like tumor. Within the tumor are the black spores, which easily get separated and dusted over the entire structure, giving it its black appearance. More than one bud in the axil may be attacked and 3, 4 or even 5 finger-like tumors can be seen emerging at one node. The scale leaves become hairy, swollen and considerably lengthened; they are filled within with the spores of the smut, the entire internal tissues being utilized by the fungus.

The masses of black spores are pulverulent and shed in enormous quantities. The galls emit the odor characteristic of wheat bunt, evidently due to the presence of trimethyl amine. The odor is so powerful that the presence of the smut can be made out even from a few feet away. The galls attract a considerable number of insects that carry the spores far and wide.

In the original description of the smut, it is stated that the sori develop in the apical region, converting the stem, bud leaves, and panicle into a large hood-shape gall. It will be noted that the abundant material that has now become available shows that the main stem is not attacked and that the parts where the sori develop are the axillary buds usually at the base of the plant. The spore masses, far from being rust colored, are almost black *en masse*, and the spores themselves are kaiser-brown to hazel (Ridgway). They are 16.0 to 23.0 μ in diameter with a mean of 19.3 μ. The mean of the measurements of 50 spores of the type specimen was reported by the writer (1940) to be 20.6 μ, but measurements of a larger number of spores now indicates that it is 19.3 μ. The sterile cells are thin-walled, smooth, 22.7 to 37.0 μ in diameter, hyaline, and occur in groups of 3 to 8. The epispore is 3 to 4 μ thick, with 5- to 6-angled reticulations. The frequencies are given below:

Diameter in μ	16	17	18	19	20	21	22	23
Frequency	9	20	32	75	28	22	9	5 = 200

Mean = 19.3 μ

Tilletia tumefaciens Sydow, loc. Rohtak, 27–12–1942, leg. S. Ahmad.

As far as can be ascertained from available literature, about 98 species of *Tilletia* have been described and, with very few exceptions, a majority of them are ovaricolous. In some cases the attacked ovaries are considerably swollen, but the formation of large tumors appears to be a character-

istic of *Tilletia tumefaciens* alone. Ciferri (1, 2) limits this genus to the ovaricolous smuts and has transferred *Tilletia earlei* Griff., which is culmicolous, to the genus *Ustilago*. However, *Tilletia flectens* Lagerheim, *Tilletia olida* (Riess) Winter, *Tilletia sterilis* Ule, *Tilletia Sesleriae* Juel are some of the well-known species whose sori are formed on the leaves in the form of long stripes and, even now (3), recognized as being well-established in the genus *Tilletia*. Limiting the genus to the ovaricolous species alone ignores the fact that the separation of the genera of the Tilletiaceae from the Ustilaginaceae is based, not on the location of the sori on the host, but on the mode of germination of the spores and other morphological characters.

The size and the general morphology of the spores of *Tilletia tumefaciens*, the place where the sori occur, the odor of trimethyl amine associated with it, and the mode of germination by formation of a nonseptate promycelium, at the apex of which the sporidia are borne, leaves no doubt that the limitation placed on the genus is not justified.

Specimens have been deposited in the Herb. Crypt. Ind. Orient.; Herb. Kew; Farlow Herbarium; and the Mycological Collections of the U. S. Dept. Agr., Washington, D. C.

SUMMARY

This note reports the rediscovery of the remarkable gall-forming species of *Tilletia* on *Panicum antidotale*, which Sydow named *Tilletia tumefaciens*. The limits of the genus *Tilletia* are discussed and evidence supplied to demonstrate that it cannot be confined to the ovaricolous smuts alone.

HERB. CRYPT. IND. ORIENT,
IMPERIAL AGR. RES. INST.,
NEW DELHI, INDIA.

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BOOK REVIEW

AINSWORTH, G. C., AND G. R. BISBY. *A Dictionary of the Fungi.* The Imperial Mycological Institute, Kew, Surrey, England. 359 pp. 138 figs. (ten plates). 1943. Price, $4.60.

It is now some years ago when I first learned of the intention of the Director of the Imperial Mycological Institute to publish a dictionary of the fungi—a sort of compendium volume to J. C. Willis' "A dictionary of the Flowering Plants and Ferns" (Cambridge, Univ. Press). All those familiar with Willis' very useful dictionary will agree that a similar one for the fungi would meet with a great reception, provided the task were accomplished in an equally creditable manner.

The book finally arrived and it affords me real pleasure to direct the attention of a wide circle of readers to same. Its aim has been nobly achieved, and, testing it from many angles, I have had no single disappointment. It is very much up-to-date. The generally unsatisfactory state of classification of fungi must have constituted quite a problem to the authors, who have, however, succeeded in not involving themselves—and the reader—in difficulties. It is surprising to note the vast amount of information the authors have been able to crowd into so little space. Many brief notes on subjects like: methods—bacteria—Ustilaginales—sex—pigments (chosen at random) have principal literature references added.

Last, but not least, the book is well got up—a credit to the printer and publisher, especially in these war times. It can be recommended without reservation as filling, most satisfactorily, a long-felt want. The authors are to be congratulated.— H. T. GÜSSOW, Associate Director, Science Service, Dominion Dept. of Agriculture, Ottawa.

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